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THE LOW WEIGHT GROUPS AND HAEMODIALYSIS

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In pediatrics haemodialysis is an accepted treatment in states of renal and prerenal fluid imbalance due to either organic or toxic causes (1 5) But the low weight groups—babies and infants—are rarely included in published series (2–4) One disincentive has been that the instrumentation is designed primarily for adults another that the tiny vessels make it exceedingly difficult to carry out the procedure on the smallest infants Fine et al have reviewed 25 haemodialyses performed on children smallest of whom weighed only 8 kg (1) In this infant cannulation was done via the femoral artery and saphenous vein for Quinton arteriovenous fistula Vein to-vein cannulation has been recommended earlier as the method of choice in the lowest weight groups (5) The main indications have been acute renal failure and intoxications This report presents experiences gained when using the low flow system and cannulation of the main veins

METHODS

Dialyser A disposable parallel flow countercurrent dialyser was used The membrane material was Cuprophane PT 300 and the dialysing surface ca 0 3 m

Delivery system The mobile type has proved practicable in the normal ward requiring no special preparations or alterations in ward construction A low dialysate flow with sufficient dialysis effect is achieved with a recirculation pump and accentuated pulsation in the membrane space caused by the piston pump The concentrations and instrument settings used for the dialysate side are presented in Table 1

Ultrafiltration

In some preliminary in vitro studies a bag containing 500 g of plasma (5 g/100 ml protein) simulated the patient The weight of the bag was monitored throughout the dialysis Various negative pressure settings were tested Fig 1 shows that within these osmolality ranges ultrafiltration is linear Ultrafiltration is also directly correlated with the dialysing surface area The line fits the equation $y = 0\,45\ x + 20\,7$ when the dialysing surface is ca 0 3 m This is in good accord with the in vivo results of Kulatilake et al They used the same type of dialyser on adult patients and calculated the ultrafiltration per square metre of dialysing surface area (6)

Extracorporeal circulation The diagram (Fig 2) shows the usual way of using a blood pump on the outlet side only (dotted line on the inlet side) to control the flow Because of the low pressure on the venous side and the small bore of the cannula it is necessary to use a pump to overcome the resistance We have used the same roller pump to pump both from and to the patient The pump tubing used is 4 mm/7 mm silicone rubber

Cannulation

Several types of cannulas have been tested The most convenient material for prolonged catheterisation has been found to be silicone rubber The blood flow range in vivo with the pump modification used is illustrated in Fig 3 The cannulas are 60 cm in length and the bore size for the smallest ones 1 02 mm Φ and 1 57 mm Φ when possible The cannulation route has been via either the jugular or the femoral vein to the caval vein and outlet up to the right atrium

Weight both during dialysis and between treatments is continuously monitored with a high accuracy electronic balance

THE PATIENTS

The series consists of seven patients (Table 2) The cases with congenital nephrosis already

Table 1 *Technical data of the pressures flow and concentrations on the dialysate and blood sides*

Dialysate				
Concentrations	Na^+ 130 mEq /l		Ca^{++}	3 0 mEq /l
	K^+ ~3 mEq /l		Mg^{++}	1 5 mEq /l
	Cl^- 101 5–104 5 mEq /l		CH_3COO^-	35 0 mEq /l
	Glucose 2 00–3 00 g/l		pH	7 4
Flow	120–150 ml/min			
Negative pressure	0–150 mmHg			
Blood				
Flow	20–50 ml/min			
Pressure in filter	0–50 mmHg			
Pressure fall	30–10 mmHg			
Priming volume				
Negative pressure		0 mmHg	108 ml	
		100 mmHg	126 ml	
		200 mmHg	136 ml	
Continuous heparinisation about 2 mg/h				
Duration				
4–6 hours				

manifested in utero are chronic cases with an otherwise fatal outcome Prolonged dialysis after bilateral nephrectomy has been the pre treatment for the planned kidney transplantation which has not succeeded so far The patient P T has been more than 5 months under dialysis The same cannulas do the work continuously The cannulas are kept open with an infusion pump In most cases, 4 hour dialysis every second or every third day combined with diet keeps the uraemia under control Even in acute cases either postoperative or tubulo circulatory which present a grave catabolic state a haemodialysis on a vein to vein basis controls the situation as shown in Fig 4 by the urea N slope One of the HUS is totally without sequelae the other as well as the acute tubular necrosis patient have slight proteinuria but are otherwise symptomfree In our three patients on whom prolonged dialysis has been performed every second or third day the treatment controls the uraemia well as indicated by the blood urea nitrogen values in Fig 5

COMMENTS

Peritoneal dialysis is a simplier method recommended for any hospital But pediatric centres

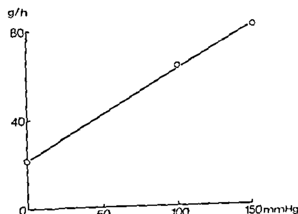


Fig 1 The registered weight loss of plasma at different negative pressure levels in vitro The equation for a 3 layer dialyser with a dialysing surface area of 0 3 m^2 is $y=(0\ 15\ x+6\ 9)\ 3$

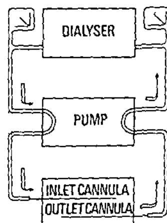


Fig 2 The extracorporeal blood circulation The tube indicated with a dotted line shows the conventional route for dialysis

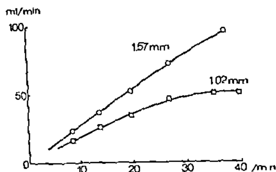


Fig 3 The effect of cannula size on blood flow The length of the cannula is 60 cm the inner diameter either 1 02 or 1 57 mm The suction is caused by a roller pump and silicone rubber tubing size 4/7 mm

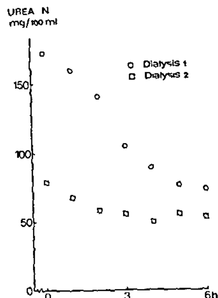


Fig 4 The blood urea disappearance from the patient during two dialyses

should have a still better method of treating cases showing acute catabolic states or in toxication The system should not be subject to any weight limitations nor should the artificial kidney need any special dialysis unit to fit into the ward surroundings This has been the basis of the policy adopted in this hospital The mobile unit has proved to be practicable Disposable dialysers with surface areas of about 0 3 (3 layers) and 0 6 m (6 layers) have been used the former for infants up to 10 kg body weight and latter after that Metcoff and others have already recommended use of the vein to vein system in acute cases in the low weight groups (5) We have found it possible

Table 2 *The treated infants*

Patient	Age (mo)	Weight (kg)	Surface area (m²)	Diagnosis	Indication	No of dialysis	Outcome
R A	17	12 6	0 52	Tubular necrosis	Anuria Hyperpotassemia Hypertension	2	Recovery
L J	24	11 5	0 50	Stenosis inf et valv a pulm.	Anuria post operat	1	Recovery
A M P	13	9 8	0 44	Hemol uremic syndrome	Anuria	5	Recovery
H P	18	11 4	0 49	Hemol uremic syndrome	Anuria	2	After 3 months residual haematuria + proteinuria
T K	2	4 0	0 23	Cong. nephrosis	Bilateral nephrectotomy	6	Died of septic infection
J G	23	6 8	0 3[illegible]	Cong nephrosis	Bilateral nephrectomy	9	Died after a transplantation attempt
P T	4 5[a]	3 2[a]	0 21	Cong nephrosis	Bilateral nephrectomy	50+	Under regular dialysis

Values at bilateral nephrectomy

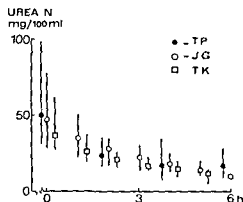


Fig 5 The means and the limits of blood urea N during haemodialysis of three bilaterally nephrectomized congenital rephrosis patients J G 9 dialyses T K 6 dialyses and P T over 50 dialyses (still continuing)

even in prolonged dialysis The priming volume of 108 to 136 ml necessitates the use of blood to fill the dialyser and to avoid a fall in haemoglobin after dialysis

SUMMARY

An adaptable artificial kidney set up for a pediatric hospital is presented No special dialysis unit is needed although the personnel must have the opportunity to keep in practice Vein to vein cannulation makes it possible to apply the method to any weight group The treatment is suitable for both acute and prolonged use The technical modifications for patients of the low weight group and dialysis data are presented

ACKNOWLEDGEMENT

The delivery system and dialyser are manufactured by Gambro Ltd Lund Sweden We are grateful to the Datex Co Ltd for providing us with their wide range electronic patient balance which is accurate to within ±5 g over a range of 0–250 kg

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FIBRINOLYSIS IN THE FIRST YEAR OF LIFE

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Much attention has been given to the fibrinolytic system in the neonatal period (see ref 5) but not to its further development during infancy

Plasminogen has been measured with streptokinase (SK) activated serum in a caseinolytic (SK-cas) or clot lysis (SK-clot) system Oehme (19) (SK-cas) and Samartzis & Cook (23) (SK-cas) found higher values for "pro fibrinolysin in late childhood and adults than in the newborn period or infancy Quie & Wannamaker (21) (SK cas) found the plasminogen content in the first 6 weeks of life to be about half of the adult level which was reached at about 6 months of age According to Kunzer & Markel (14) the proactivator activity in cord blood is 50 of adult level and successively reaches the lower border of the range of adult activity at about 12 months of age Ambrus et al (1) (SK-clot) found the plasminogen content in the newborn serum during delivery to be about one tenth of that in adults and to increase to adult level during the first 7 months According to Bruster & Pfitzner (4) (SK-clot) the adult plasminogen range was reached at the end of the first 3 months of life

Inhibitors of fibrinolysis Earlier investigators have not distinguished between inhibitors of plasminogen activation and antiplasmin Samartzis & Cook (23) found "antifibrinolysin to be somewhat lower in 7–12 month-old infants than in adults whereas Quie & Wannamaker (21) and Ambrus et al (1) found the level of inhibitors to be the same in newborns as in adults In Bruster & Pfitzner s (4) series the values were higher in newborns and fell to adult level within the first 3 months of life Ganrot & Schersten (9) reported on the variations of α macroglobulin with age and sex They found values for cord blood to be about 1 5 times the adult value In 1–3 year old children it was 2 5 times this level and in infants 0–1 year it was somewhat lower than in the latter group

The *fibrinogen* has been described as slightly below (24 26 27) or at adult level (22 25) during the first week of life but after this period it does not differ significantly from adult level (22 24 25 26 27) As far as we know Uttley et al (26) were the first to study fibrin/fibrinogen degradation products (FDP) in healthy infants They collected the blood in tubes containing an inhibitor of fibrinolysis Using the immunoassay method of Merskey et al (15) they found an overall level of about 11 μg/ml in 0–12 year-old children This level was somewhat higher than in adults and the highest levels were found in the 1 week to 1 year age group

This study is a link in a serial investigation of fibrinolysis from early foetal life up to the first year of life (5 6)

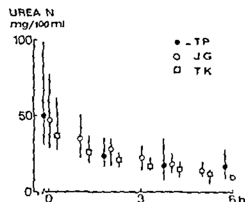


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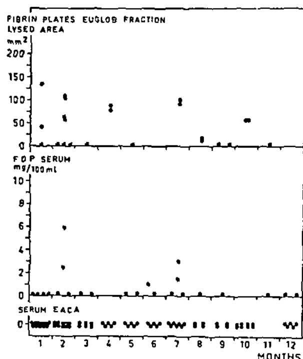


Fig 1 Fibrinolytic activity on unheated fibrin plates (resusp euglobulin precipitate) in 41 infants Fibrin/fibrinogen degradation products (FDP) in serum and serum EACA from 60 infants and in only serum EACA from 13 other infants

MATERIAL AND METHODS

Blood samples were obtained from 82 10 day to 12 month old apparently healthy infants in the departments of Paediatrics Paediatric Surgery Plastic Surgery and a children s home They had had no recent infections and the haemoglobin values and microsedimentation rates were within normal limits None of the infants had been vaccinated within the last 2 weeks before sampling The infants had been admitted because of simple feeding problems inguinal hernia cleft lip and palate (but otherwise no complex malformations) and on sociomedical grounds

Blood sampling

Blood from a puncture of the cubital vein was allowed to flow freely into test tubes or was withdrawn with a disposable plastic syringe from a femoral vein It was not always possible to obtain samples large enough for the complete set of determinations

Laboratory procedures

The blood was collected in siliconized glass tubes and in disposable plastic tubes Citrated plasma and serum were prepared in the way described previously (17 20) The following determinations were made

1 Fibrinolytic activity of plasma and resuspended euglobulin precipitate fibrin plate method (2)

2 Fibrin/fibrinogen degradation products (FDP) in serum and in serum from blood collected in a tube with aminocaproic acid (EACA) immunochemical method (16)

3 Plasminogen immunochemical method (5 8)

4 α-macroglobulin esterolytic method (7)

5 Antiplasmin activity (progressive antiplasmin") fibrin plate method (6)

6 Inhibitors of plasminogen activation clot method (20)

7 Fibrinogen spectrophotometric method (18) In this method 9 parts of blood are added to 1 part of 3 8 citrate solution and 1 part of citrate EACA solution The dilution of blood is thus 2 11 Correction for plasma dilution at various haematocrit (hct) values is obtained by a factor given in the formula $\frac{9(1-hct)+2}{9(1-hct)}$ For a hct of 40 % this factor is 1 37 for hct values of 60% and 30% it is 1 56 and 1 43 respectively

8 The haematocrit was determined with a microcapillary centrifuge (Cellokrit AB Lars Ljungberg & Co Stockholm)

RESULTS

Fibrinolytic activity on fibrin plates (Fig 1) was measured in 41 infants The areas lysed by plasma and euglobulin fraction on unheated plates (indicating plasminogen activator activity) exceeded the normal adult range (0–82 and 0–159 mm², respectively) in only 2 cases In 8 infants no activity at all could be demonstrated Only the values for euglobulin fraction are given in the figure The plasma showed no plasmin activity except in one infant (25 mm) whereas the euglobulin fraction exhibited a weak activity in 15 (11–35 mm) The fibrinolytic activity did not vary with the age of the infants

Fibrin/fibrinogen degradation products

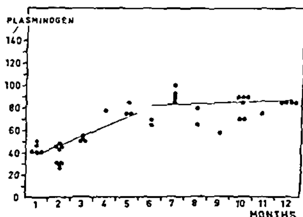


Fig 2 Determinations of plasminogen in 71 infants

(*FDP*) were assayed in serum and in serum EACA Pairs of samples were obtained from 60 infants In another 13 infants determinations were made only in serum EACA The results are given in Fig 1 42 infants had FDP in serum ranging from 1 0 to 9 0 mg/100 ml whereas the corresponding assays in serum EACA were negative Another 18 infants had no demonstrable FDP in the serum or in serum EACA Serum EACA from 13 infants studied contained no demonstrable FDP

Plasminogen (Fig 2) was determined in 76 infants A simple linear regression analysis revealed a significant increase during the first 5 months ($y = 8\,3\,X + 30\,7$ $r = +0\,59$ $p < 0\,01$) After this period all values except 3 were within the lower border (61 %) of the normal adult range

α macroglobulin (Fig 3) showed no significant change with age in the 59 infants studied

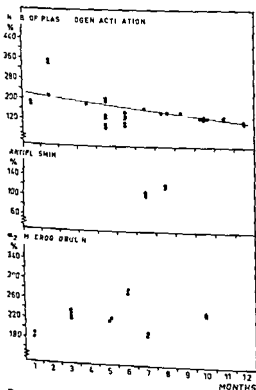


Fig 3 Determinations of the *inhibitors* of fibrinolysis α_2-macroglobulin in 59 infants antiplasmin *in* 30 infants and the inhibitors *of* plasminogen activation in 59 infants

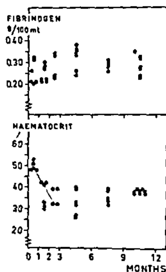


Fig 4 Fibrinogen in 54 infants and haematocrit in 52 infants

($r = +0\,18$) The mean and S D was 228 ± 36

Antiplasmin activity (Fig 3) was measured in 30 cases No significant change with age was found ($r = +0\,10$) The mean and S D was 105 ± 16 %

Inhibitors of plasminogen activation (Fig 3) studied in 59 infants showed a significant decrease with increasing months of life The regression equation was $Y = 225\,3 - 8\,7\,X$ ($r = -0\,40$ $p < 0\,01$) The values thus fell from the moderately higher levels in the newborn to normal adult range within this age period

Fibrinogen (Fig 4) corrected to the haematocrit changes showed no significant changes ($r = +0\,16$) The mean and S D for 54 infants was 0 28 ± 0 05 g/100 ml

Haematocrit (Fig 4) was estimated in 52 infants There was a significant decrease during the first 3 months of life The regression equation was Y 56 9 − 8 2 X ($r = -0\,78$ $p < 0\,01$)

DISCUSSION

Earlier investigations at this laboratory (5 6) have shown that in human foetuses and full term newborns the fibrinolytic capacity is

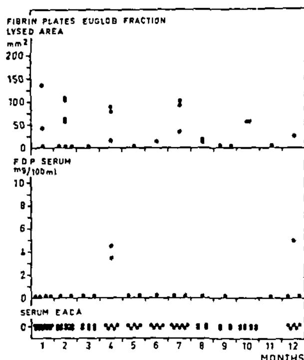


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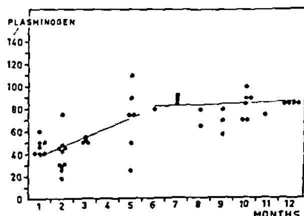


Fig 2 Determinations of plasminogen in 71 infants

fections sometimes complicated by disseminated intravascular coagulation Primary fibrinolysis may also occur as in cyanotic congenital heart disease (3) Knowledge about the fibrinolytic system in healthy infants is therefore of importance as a basis for further studies of pathological conditions

SUMMARY

The fibrinolytic system was studied in 82 healthy infants Except plasminogen the various factors reached adult level already in early infancy The plasminogen activator content was found to be sufficient in most infants and compensated for the lower plasminogen values during the first half year of life Inhibitors of plasminogen activation were higher at birth and fell to normal adult level during infancy The results may serve as a basis for further studies in diseases prone to be complicated by disseminated intravascular coagulation or primary fibrinolysis

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higher, the plasminogen content is lower, and the levels of the inhibitors of plasminogen activation higher than in adults α_2 macroglobulin increases significantly during foetal life to reach values above adult level in the fullterm newborn, whereas antiplasmin is at adult level throughout foetal life and in the new born

This study deals with the pattern of the fibrinolytic system during the first year of life

Determination of the fibrinolytic activity on fibrin plates hitherto not used in studies in infancy, was found to fall within the normal adult range This result is in agreement with that obtained by Stroder & Kunzer (24), who used a fibrinogenolytic method

Plasminogen activator activity was also measured as the difference in amount of FDP in serum and in serum with an inhibitor of fibrinolysis (serum EACA) 42 out of 60 infants showed such activity No FDP could be demonstrated in serum EACA With the method introduced by Nilehn (16) FDP can be demonstrated down to a concentration of 5 μg/ml The method has proved valuable FDP never being demonstrable in health but always denoting disease when present in amounts exceeding the above critical level These findings have since been confirmed at our laboratory in extensive studies of more than 3 000 adults (11) Uttley et al (26) found FDP to be on the average 10 7 μg/ml during the first week of life and 14 0 μg/ml from 1 week to 1 year of age The normal adult range for their method is 0–10 μg/ml Since it is questionable whether the small amounts of FDP above the normal adult range are of any practical importance their results may be regarded as compatible with ours

Plasminogen was assayed with an immunochemical method permitting direct determination The blood sample is collected in tubes containing EACA which inhibits spontaneous *in vitro* activation of the plasminogen The results obtained with this method are closely correlated with those obtained with a caseinolytic method in which plasminogen is activated by urokinase and the inhibitors precipitated with ammonium sulphate (12) As mentioned in the introduction all earlier determinations of plasminogen in infancy have been made in streptokinase activated serum, which is less reliable because of the possible presence of streptokinase antibodies in the serum However, the changes in plasminogen assayed with our method agreed fairly well with those found in earlier investigations, i e the plasminogen level is low in the newborn but reaches the adult range within the first 5–6 months

As to antiplasmin activity the total plasmin inhibiting effect of serum has been found to be of adult strength by all investigators except Bruster & Pfitzner (4) who reported the newborn antifibrinolysin to be higher and to fall to normal adult level within the first three months We found a normal adult content of the progressive antiplasmin throughout infancy Hitzig (13) was the first to report quantitative determinations of α_2 macroglobulin in the post natal period and found higher values throughout infancy with a mean clearly higher than in adults Our results show a two fold adult content of α_2-macroglobulin compatible with those given by Ganrot & Schersten (9) They are largely in agreement with those of Hitzig but are not strictly comparable if values are given in mg/100 ml because of the use of different standard serum pools

Inhibitors of plasminogen activation have not previously been studied in infancy In a recently published study (5) we found moderately higher values in newborns than in adults Our present findings show that this change occurs during infancy with a successive fall within the first 12 months

As to fibrinogen no significant changes could be demonstrated throughout infancy which is in accordance with earlier studies (22 24 25 26 27)

Diseases of infancy with involvement of the fibrinolytic system are by no means rare Hathaway (10) has recently given a survey of conditions such as severe bacterial or viral in

fections sometimes complicated by disseminated intravascular coagulation Primary fibrinolysis may also occur as in cyanotic congenital heart disease (3) Knowledge about the fibrinolytic system in healthy infants is therefore of importance as a basis for further studies of pathological conditions

SUMMARY

The fibrinolytic system was studied in 82 healthy infants Except plasminogen the various factors reached adult level already in early infancy The plasminogen activator content was found to be sufficient in most infants and compensated for the lower plasminogen values during the first half year of life Inhibitors of plasminogen activation were higher at birth and fell to normal adult level during infancy The results may serve as a basis for further studies in diseases prone to be complicated by disseminated intravascular coagulation or primary fibrinolysis

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LACTOSE MALABSORPTION IN FINNISH CHILDREN OF SCHOOL AGE

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From the Department of Public Health Science the Gastroenterological Laboratory Second Department of Medicine and the Children s Hospital University of Helsinki and Public Health Research Centre National Board of Health Helsinki Finland

Recent studies have shown that lactose malab sorption (LM) due to lactase deficiency varies in frequency in different populations from 2 6–6 6 % in Danish gastroenterological pa tients (8) to 100 % in healthy adult Thais (6) However there are few investigations of the age at which lactase deficiency appears in dif ferent populations Virtually all the Thai chil dren older than 2–4 years who were studied had isolated LM (6 13) and in Bantu tribes in Uganda LM seems to appear very early (5) Recently Bolin and his co-workers (4) showed that LM is universal above 10 years of age in Singapore

In an earlier study by our group the preva lence of LM in a Finnish rural population from 21 to 65 years of age was 15 % (12) As the prevalence was similar in all age groups and congenital LM is extremely rare 7–15 year-old children from the same community were studied to discover the prevalence of LM in children of school age and the age at which LM appears We also noted abdominal com plaints and the effect of a lactose free diet to get a picture of the practical significance of LM

MATERIAL AND METHODS

Pornainen in Southern Finland is a small rural community the main occupation in which is agri culture (about 60%) One-quarter of the inhabitants cam as evacuees from Karelia during the Second World War

In 1969 the total number of 7–15 year-old children in Pornainen was 380 162 of these were selected by simple random sampling The families were sent a qu stionnaire concerning the children s abdominal complaints and food habits The children were all invited to undergo an examination to detect func tional disorders of the small intestine Of the children selected 11 had moved from the community and of the remaining 151 children the questionnaires were returned by 150 Of the e 130 (60 boys and 70 girls) were willing to participate in th examination i e 80% of the 162 children selected There was no statistically significant difference between thos who participat d and those who declined examination as regards age sex milk drinking habits and occurrence of abdominal complaints

Lactose tolerance test (LTT)

After overnight fasting 1 g/kg of lactose was given as a 12 5% solution the maximum dose being 50 g Capillary b ood samples were taken before and 20 and 40 min aft r lactose ingestion The glucose con centration was determined by the glucose oxidase method (10) Abdominal symptoms on the day of LTT were noted A maximum rise in blood glucose con centration of 25 mg/100 ml or more was taken as a sign of normal lactose absorption Th children who had a borderline rise between 20 and 24 mg/100 ml had a second LTT with double doses of lactose 2 g/ kg Those with a maximum rise of 20 mg/100 ml or more in this repeated LTT were considered to have normal lactose absorption

Chi dren with maximum rises of less than 20 mg/ 100 ml in the first or repeated LTT were sent for a small intestinal biopsy to the outpati nt department of the Children s Hospital University Central Hospital of Helsinki Biopsy samples were examined with a dissection microscope and histologically and the mu

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Children with maximum rises of less than 20 mg/100 ml in the first or repeated LTT were sent for a small intestinal biopsy to the outpatient department of the Children s Hospital University Central Hospital of Helsinki Biopsy samples were examined with a dissection microscope and histologically and the mu

Table 1 *Findings in lactose tolerance tests histology, lactase activity and lactase sucrase ratio of small intestinal mucosa in children with rise in blood glucose concentration of less than 20 mg/100 ml in LTT*

Subject		Age	Sex	Maximum rise in blood glucose concentration in LTT (mg/100 ml)	Small intestinal histology	Lactase activity[a] (U/g protein)	L/S[b]	Maximum rise in blood glucose (A) and galactose (B) concentrations in LTT with ethanol	
								A	B
Group A lactose malabsorption confirmed									
1	EM	15	F	0	—	—	—	1	<1
2	TP	13	F	7	Normal	9 4	0 06	—	—
3	JN	8	M	7	Normal	16 6	0 13	—	—
4	JL	13	M	11	—	—	—	7	2
5	JK	8	F	13	Normal	4 6	0 07	—	—
6	SLe	13	F	14	Normal	—	—	19	4 5
7	SLa	11	M	16	Normal	6 3	0 10	—	—
8	RJ	14	M	16	Normal	4 2	0 09	—	—
Group B lactose malabsorption excluded									
9	SLu	9	M	8	Normal	56	0 41	—	—
10	HN	14	M	13	—	—	—	30	20
11	SS	12	F	17	Normal	53	0 71	—	—
12	LK	7	M	17	Normal	74	0 76	—	—
13	SM	13	F	21/17[c]	Normal	24	0 33	—	—

[a] Normal values 20 U/g protein or more
[b] Normal values 0 30 or more
[c] In repeated LTT

cosal maltase sucrase and lactase activities were determined as described earlier (15) The disaccharidase activities were calculated against the protein content of mucosa measured by the method of Lowry et al (16) With the method used normal values for lactase activity are 20 U g protein or more and for lactase/sucrase ratio 0 30 or more as calculated from the adult material presented by one of us earlier (11)

Two children refused small intestinal biopsy and in one child the biopsy failed In these children the lactose tolerance test with ethanol administration was performed as described earlier (11) They were given 0 3 g/kg ethanol which inhibits the metabolism of galactose in the liver (20) and 15 min later 1 g kg lactose as 12 5 solution Capillary blood samples for determination of glucose and galactose concentrations by the glucose-oxidase (10) and galactose-oxidase (9) methods respectively were taken before and 20 and 40 min after ingestion of lactose

The normal absorption of monosaccharides in all children with low rises in the LTT was confirmed by the glucose-galactose tolerance test (GGTT) giving 0 5 g/kg of glucose and 0 5 g/kg of galactose

Criteria for selective lactose malabsorption (SLM)

The criteria were that the child had (*a*) a maximum rise of less than 20 mg/100 ml in the LTT and (*b*) normal small intestinal histology lactase activity less than 20 U/g protein and lactase/sucrase ratio less than 0 30 or in the LTT with ethanol administration had a maximum rise in blood glucose concentration of less than 20 mg 100 ml and in galactose concentration of 5 mg/100 ml or less

Statistical methods

The Chi square test (7) was generally used Whenever it was not suitable the Fisher s exact probability test was used (7) The differences were considered statistically significant if the *p* value was less than 0 05

Lactose free diet

Children with both SLM and abdominal complaints were put on a lactose free diet and the effect of the diet on abdominal complaints and stool frequency was estimated subjectively after from 2 weeks to 1 month During the lactose free diet period foods containing milk and lactose were forbidden but cheese butter and sour milk products were allowed

RESULTS

Of the 130 children studied 14 had a maximum rise in blood glucose concentration of less than 20 mg/100 ml in the LTT Of these one who gave no history of abdominal complaints

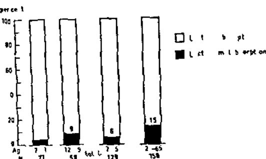


Fig 1 The prevalence of lactose malabsorption in Finnish children of school age and in the adults from the same community studied earlier (12)

and had no symptoms after the LTT refused the further studies and was thus excluded from the material The other 13 children with low rises in the LTT all had increases of more than ?0 mg/100 ml in the GGTT In 8 of these intestinal lactase deficiency and selective lactose malabsorption could be demonstrated by determination of mucosal lactase activity or the LTT with ethanol administration (Table 1) In five others no lactase deficiency was demonstrable and the primary LTT had been misleading Thus *in our series* 8 out of 129 school children had SLM This means a prevalence of 6 which was the same in boys and girls In the age groups from 7 to 11 years and from 12 to 15 years the prevalence was 4 and 9 respectively (Fig 1) This difference is not however statistically significant Comparing this prevalence with that (12) found earlier in the same community in adults (15%) the difference is statistically significant (χ =5 806 df=1 0 025 > p > 0 01)

Of the 8 school children with SLM all but one had drunk at least 2 to 3 glasses of milk daily since weaning (Table 2) Four of them gave a history of weekly abdominal complaints and they also had symptoms after the LTT Subjects 4 and 6 who had no symptoms after the LTT had however meteorismus after the LTT with ethanol administration Subject 4 also gave a history of meteorismus occurring every second week None of the children with a low rise in the LTT but with normal lactase activity and lactose absorption gave a history of abdominal complaints or had symptoms after the LTT Of the 121 children with normal lactose absorption 20 (17%) had weekly abdominal complaints (Table 3) The difference in frequency of symptoms between children with SLM and children with normal lactose absorption was not however statistically significant Taking the abdominal complaint singly the children with SLM had weekly meteorismus significantly more often than those with normal lactose absorption After the LTT the SLM children had symptoms significantly more often than children with normal lactose absorption

Table 2 *Milk habits abdominal complaints and the effect of lactose free diet on abdominal complaints in 8 children with lactose malabsorption*

			Average milk drinking glasses per day		Abdominal complaints		Effect of lactose free diet on abdominal complaints		
Subject	Age	Sex	Present	After weaning	Previous	After LTT	Dis appeared	Diminished	Unchanged
1 EM	15	F	1–3	2–3	P M E	P L N	P M E		
2 TP	13	F	1 3	2 3	None	None			
3 JN	8	M	1–3	2–3	None	None			
4 JL	13	M	Over 5	2–3	None	None			
5 JK	8	F	1 3	2 3	L M	P L	L M		
6 SLe	13	F	3–5	2 3	None	None			
7 SLa	11	M	1 3	2 3	P L, F N E M	P M	L N M		P F E
8 RJ	14	M	3–5	1					

P = pain, L = loose stools M = meteorismus F = fullness N = nausea E = eructation
Complaints occurring at least once a week are given

Table 3 *Frequency of various abdominal complaints in 8 children with selective lactose malabsorption and in 121 children with normal lactose absorption*

	Lactose malabsorption (n = 8)		Lactose absorption (n = 121)	
	Previous[a]	After LTT	Previous[a]	After LTT
Pain	2	3	13	9
Loose stools	2	1	3	2
Watery diarrhoea	0	1	0	0
Meteorismus	3[b]	1	7[b]	5
Fullness	2	0	3	0
Nausea	1	1	4	3
Eructation	2	0	6	0
Heartburn	0	0	2	0
Total number of children with abdominal complaints	4	4[c]	20	16[c]

[a] Complaints occurring at least once a week are given
[b] Difference is statistically significant ($p = 0.031$ Fisher s exact probability test)
[c] Difference is statistically significant ($p = 0.040$ Fisher s exact probability test)

In all children with SLM and abdominal complaints the lactose free diet removed or diminished the symptoms in from 2 weeks to 1 month (Table 2) In subject 7 pain fullness and eructation also lessened over 2–3 months

Taking the material as a whole of the 129 children studied, 24 (19%) had weekly abdominal complaints of which 15 (12%) also had recurrent abdominal pain The latter is of the same magnitude as that found by Apley (10 8%) in his extensive study of school children (1) In one sixth (4 out of 24) of all children with abdominal complaints SLM was probably the main cause of symptoms as indicated by the beneficial effect of a lactose free diet on symptoms Thus SLM has clear practical significance

The birth weight of children with SLM had been normal (mean 3 560 g range 2 800–4 520 g) and their present height and weight did not differ from those of children with normal lactose absorption

DISCUSSION

This study shows that SLM is already present at school age in a Finnish rural population the prevalence being 6% Prevalence tended to increase with age (Fig 1) so Finns may get LM before school age but it appears mostly between 10 and 20 years This is obviously much later than in the children of Thailand (6 13) and Singapore (4) all of whom had LM by the age of 4 and 10 years respectively

Two different theories for the aetiology of SLM have been presented Bolin & Davis (3) put forward a theory of lactase adaptation They found (4) a relationship between lactose tolerance and continued milk intake after weaning The prevalence of SLM was highest in those who had taken milk for the shortest period after weaning They concluded that *the lack of substrate stimulation is the main* cause of lactase deficiency and SLM The other theory is that genetic factors have a basic role in the aetiology of SLM (2 18 19) In our material all children with SLM had drunk milk daily regularly and in the same amounts as the children with normal lactose absorption This means that a lack of substrate stimulation is not the cause of SLM in our material There is preliminary data showing in the Finnish population that there is a clear familial *clustering in the occurrence of SLM* and that there is no difference in milk drinking habits between those who have and those who do not have SLM This means perhaps that genetic factors have a central role in the aetiology

of SLM The fact that SLM appears later in Finns than in Thais for instance may be due to the regular and abundant use of milk (290 kg per capita during 1965) in the Finnish population We can assume that the basic aetiological factor in SLM is genetic but that the dietary habits may greatly influence the age at which SLM is manifested

The occurrence of weekly abdominal complaints in children with SLM and in children with normal lactose absorption was 50 and 17 respectively Meteorismus fullness and loose stools were certainly more common in children with SLM than in children with normal lactose absorption but only with meteorismus was this difference statistically significant The smallness of the material probably explains this After the LTT SLM children had symptoms significantly more often than children with normal lactose absorption (50 and 13 % respectively)

In an earlier investigation (12) in adults in the same community it was found that more than 50 of subjects with SLM gave a history of weekly abdominal complaints The symptoms were more frequent in older than in younger subjects After the LTT only 5 of 27 adults with SLM had no abdominal symptoms Thus it appears that children with SLM are more often symptomless than adults with SLM and that the symptoms in SLM become more common with increasing age The probable explanation for this is that the colon of healthy children and young adults does not easily react with increased peristalsis to the increased intraintestinal volume of fluid caused by unabsorbed lactose in the small intestine (14) In the colon lactose is split by bacterial fermentation and water and electrolytes can be absorbed

In present series the LTT gave false positive results in 5 out of 13 children The same observation was made in another series of children (Launiala & Sahi unpublished data) In some cases the flat curve in the LTT of children with normal lactose absorption may be due to the rapid utilization of glucose reflected also in a flat curve in the glucose-galactose tolerance test However, in most cases the cause was obscure but the emptying of the stomach might influence this (17) Using ethanol as an inhibitor of galactose metabolism in the liver it is possible to follow also blood galactose concentrations after lactose administration This gives more reliable information about intestinal splitting of lactose Because the amount of ethanol needed for this inhibition (0 3 g/kg body weight) does not cause noticeable symptoms the test is very useful in children However more investigation is needed before using this test in infants and small children

SUMMARY

Lactose absorption and the occurrence of weekly abdominal complaints was studied in randomly selected 7–15 year-old children of a Finnish community A peroral lactose tolerance test was first performed If the maximum rise was less than 20 mg/100 ml a peroral small intestinal biopsy and determination of mucosal maltase sucrase and lactase activities were made or a peroral lactose tolerance test with ethanol administration was done

Selective lactose malabsorption (SLM) was found in 8 of the 129 children studied (prevalence 6 %) The prevalence was the same in boys and girls and in two age groups 7–11 and 12–15 years it was 4 and 9% respectively This difference was not statistically significant

Weekly abdominal complaints occurred in 50 of children with SLM the main symptoms being meteorismus loose stools and fullness and in 17 of children with normal lactose absorption the main symptoms being abdominal pain meteorismus and eructation In the SLM children meteorismus was however significantly more common than in children with normal lactose absorption

This prevalence of SLM in children of school age was compared with that found in adults in the same community It appears that SLM begins before the age of 7 years but that

it most commonly develops between 10 and 20 years

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CHROMOSOME STUDIES IN 30 CHILDREN WITH TURNER S SYNDROME

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Numerous reports already exist on the chromosomal patterns in patients with Turner s syndrome Most of these are case reports but larger series of patients have also been pub lished (1 2 4 8) In most of these patients the d agnosis Turner s syndrome has been made after the age at which puberty normally occurs The suspicion of Turner s syndrome in children is often based upon dwarfism com bined with webbed neck antimongolian slant ing of the eyes etc whereas the cardinal symp toms in adults may be primary amenorrhoea and more or less incomplete pubertal develop ment One of the purposes of the present work was therefore to examine chromosomal pat terns in a larger number of patients in whom the diagnosis of Turner s syndrome was made in childhood to see if the chromosomal pat terns of these patients differed from the group of patients in whom the diagnosis was made af ter the age of normal puberty Furthermore it was found to be of interest to examine the present series of patients with regard to Xg bloodgroup system

MATERIAL AND METHODS

The diagnosis of Turner s syndrome has been made on usual clinical criteria and hormone analysis Later we want t) report in detail the different clinical roentgenologi al and laboratory findings in these pa tients compared with th ir chromosomal patt rns The patients have all been diagnosed and followed many of them for years in the Endocrine Clinic at the Queen Louise Childrens Hospital in Copenhagen

The chromosomal studies were carried out from 1964 to 1967 The karyotype was studied on blood cultures prepared according to Frøland s method (3) a micromethod based on th principles of Moorh ad et al (7) Nuclear sex was determined on buccal smears stained by the Feulgen method In 2 patients additionally skin culture prepared at the Institute for Human Genetics at the University of Cop nhagen was studied

In 23 ca es it was possible to make blood group determination on patients and both parents The b ood group ABO Rh sus MNS and I system were carried out at the State Serum Institute in Copenhagen and since they were not contributcry they will not be further mentioned Xg blood group determinations w re performed at the Lister Institute in London

In the following the nomenclature of chromo omal abnormalities accepted at the Chicago Conference in 1966 is used

This investigati n was made possible by grants from P Carl Petersens Fond

RESULTS

Table 1 shows the karyotype the distribution of chromosome counts the total number of cells counted and the percentage of sex chro matin positive cells 15 of the patients (50%) had karyotype 45 X without mosaicism and all were sex chromatin negative 3 patients had diploid modal count—in one 46 XX (292) was found (apparently normal female karyotype) and in two others were found 46 XXqi in whom the abnormal chromosome w is assumed to be an isochromosome for the long arm of the X chromosome All three were sex chromatin positive In 12 patients (40 %) the chromosomal examination showed mosaicism in

Table 1 *Karyotype distribution and chromosome counts and sex chromatin in 30 patients with Turner syndrome*

Xqi = isochromosome for the long arm of the X chromosome Xr ringchromosome
Xq− = deletion of the long arm of the X chromosome)

Ref no	Karyotype	Chromosome counts 43	44	45	46	47	Total cells	Sex chromatin positiv
115	45 X	4	9	45			58	4
162	—	2	5	50			57	4
174	—		4	45	1		50	8
223	—	2	2	32			36	0
226	—		2	43			45	2
228	—	1	3	55			59	2
243	—	1	2	47			50	0
249	—	3	3	35			41	1
260	—	2	3	41	2		48	5
266	—		5	43			48	0
270	—		2	49			51	0
286	—	1	2	51			54	0
289	—	3	1	46			50	1
302	—	1		39			40	2
304	—	1		39			40	4
135	46 XXqi	1	1	2	66		70	25
257	—	1		2	49		52	22
292	46 XX			2	56		58	24
51	45 X/46 XX	2	6	33	19		60	10
101	—	2	2	23	24		51	17
230	—	1	5	17	51		74	14
244	—	2		19	42	1	64	30
262	—	1	4	57	13		74	12
136	45 X/46 XXr	2	3	44	36	2	87	2
256	—	2	1	52	36		91	3
— skin	—	1	6	74	18		99	
291	—		4	98	16		118	1
130	45 X/46 XXq−	1	4	46	34		85	1
234	45 X/46 XXqi		11	149	36		196	4
100	—	3	3	36	42	1	85	16
160	45 X/46 XY	2	5	39	81		127	2
— skin	45 X	5	5	71	1	3	85	

which one cell line always was 45 X and the other cell line either normal female karyotype (51 101 230 244 262) ring chromosome for one X chromosome (136 256 291) deletion of the long arm of one X chromosome (130) isochromosome of the long arm of one X chromosome (234 100) or normal male karyotype (160) Of these 12 patients with mosaicism 6 were sex chromatin negative one positive and 5 had sex chromatin in a lower number than in normal females

In 2 patients additional chromosome examinations on skin cultures have been carried out One patient (256) had the same karyotype 45 X/46,XXr in both skin and blood cells Contrary to this the other patient (160) had 45,X/46 XY in the blood cells whereas the skin cells showed 45 X without mosaicism

Table 2 shows the results of Xg blood group determinations in 23 patients and their parents In 4 of the patients it was possible to deduce the origin of the X chromosomes In patients 223 and 249 with the karyotype 45 X the single X was of maternal origin as both patients and their mothers were Xg(+) and both fathers Xg(−) In patient 135 with the karyotype 46 XXqi and in patient 234 with 45 X/46 XXqi the normal X was of maternal origin and the isochromosome of paternal origin as both patients were Xg(−) and both set of parents Xg(+) Furthermore one may assume here that the Xg gene must be located on the

Table 2 *Yg blood type of 23 patients with Turner s syndrome and their parents and the origin of the X chromosome*

Ref no	Karyotyp	Xg blood group Xg (a)			Origin of X chromosom
		Patient	Mother	Father	
					?
115	45 X		−	+	?
174	—	+	−	−	Maternal single X
223	—	+			
278	—	−			?
243	—	+			Maternal single X
349	—	+	+		?
260	—	−	+		
66	—	+		+	?
283	—	+			?
301	—	−	−	−	Maternal X and paternal Xqi
135	46 XXqi	−	−	+	Xg^{a} must be located on the short arm of X chromosome
25	—				?
51	45 X 46 XX		−	−	?
101	—				?
30	—				?
244	—			−	
136	45 X 46 XXr				Paternal Xr ?
91	—				Paternal Xr ?
130	45 X 46 XXq			−	?
100	45 X/46 XXqi				?
234	—	−			Maternal X and paternal Xqi Xg^{a} must be located on the short arm of X chromosome
160	45 X 46 XY		−		
9	46 XX		−		

short arm of the X chromosome and that the mothers must be heterozygotes for the Xg gene In these 4 cases the conclusion could be drawn that the missing X or the abnormal X must be of paternal origin

In two more cases one may assume that the abnormal X chromosome was of paternal origin This is patients 136 and 291 both with karyotype 45 X 46 XXr as both patients and their mothers were Xg(+) whereas the fathers were Xg(−)

DISCUSSION

The X chromosome belongs according to its size to group C (chromosome 6–12) but it cannot by the usual technique be identified from the other chromosomes in the group The first 15 patients were sex chromatin negative with 45 chromosomes and a missing chromosome in group C indicating 45 X Patients 135 and 257 were both sex chromatin positive with 46 chromosomes in the cells However 3 chromosomes of the size of chromosome number 3 and only 15 chromosomes in group C could be found It seems likely therefore that these patients have an isochromosome for the long arm of one X chromosome (Fig 1)

In 3 patients (136 256 291) with mosaicism some of the cells contained a ring chromosome (Fig 2) They were all sex chromatin negative and it must be assumed therefore that this was a ring chromosome for one of the X chromosomes

Patient 130 had 45 chromosomes in about half of the cells with only 15 chromosomes in group C and in the other half of the cells 46 chromosomes with 15 chromosomes in group C and a small extra chromosome (Fig 3) She was sex chromatin negative and it may be as-

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228	—	1	3	55			59	2
243	—	1	2	47			50	0
249	—	3	3	35			41	1
260	—	2	3	41	2		48	5
266	—		5	43			48	0
270	—		2	49			51	0
286	—	1	2	51			54	0
289	—	3	1	46			50	1
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		Xg blood group Xg (a)			
Ref no	Karyotyp	Patient	Mother	Father	Origin of X chromosom
115	45 X	+	–		?
174	—	+	–		Maternal single X
273	—	+	–		?
278	—	+	+	+	?
243	—	+	–		Maternal single X
249	—	+	–		?
260	—	–		–	
266	—	–		+	?
289	—	+	–	+	?
304	—	–	–	–	
135	46 XXqi	–		+	Maternal X and paternal Xqi Xg[a] must be located on the short arm of X chromosome
257	—	–	–		?
51	45 X 46 XX		–	–	?
101	—		–	–	?
230	—	–	–	–	?
244	—	–	–	–	?
136	45 X 46 XXr		–	–	Paternal Xr ?
291	—		–	–	Paternal Xr ?
130	45 X 46 XXq	–		–	
100	45 X 46 XXqi			–	
234	—	–			Maternal X and paternal Xqi Xg[a] must be located on the short arm of X chromosome
160	45 X 46 XY	–			
29	46 XX	–	+		

short arm of the X chromosome and that the mothers must be heterozygotes for the Xg gene. In these 4 cases the conclusion could be drawn that the missing X or the abnormal X must be of paternal origin.

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DISCUSSION

The X chromosome belongs according to its size to group C (chromosome 6–12) but it cannot by the usual technique be identified from the other chromosomes in the group. The first 15 patients were sex chromatin negative with 45 chromosomes and a missing chromosome in group C indicating 45 X. Patients 135 and 257 were both sex chromatin positive with 46 chromosomes in the cells. However 3 chromosomes of the size of chromosome number 3 and only 15 chromosomes in group C could be found. It seems likely therefore that these patients have an isochromosome for the long arm of one X chromosome (Fig 1).

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Ref no	Karyotype	Chromosome counts					Total cells	Sex chromatin positiv
		<43	44	45	46	47		
115	45 X	4	9	45			58	4
162	—	2	5	50			57	4
174	—		4	45	1		50	8
223	—	2	2	32			36	0
226	—		2	43			45	2
228	—	1	3	55			59	2
243	—	1	2	47			50	0
249	—	3	3	35			41	1
260	—	2	3	41	2		48	5
266	—		5	43			48	0
270	—		2	49			51	0
286	—	1	2	51			54	0
289	—	3	1	46			50	1
302	—	1		39			40	2
304	—	1		39			40	4
135	46 XXqi	1	1	2	66		70	25
257	—	1		2	49		52	22
292	46 XX			2	56		58	24
51	45 X/46 XX	2	6	33	19		60	10
101	—	2	2	23	24		51	17
230	—	1	5	17	51		74	14
244	—	2		19	42	1	64	30
262	—	1	4	57	13		74	12
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Ref no	Karyotyp	Xg blood g oup Xg (a)			Origin of X chromosome
		Pati nt	Mot ıer	Father	
115	45 X	−	−	+	?
174	—	+	−		?
273	—	−	−	−	Maternal single X
278	—	+	−		?
243	−		−		Maternal single X
249	−	+	−	−	?
260	—	−	−	−	?
266	—		+		?
289	−	+		+	
304	−		—		
135	46 XXqi			−	Maternal X and paternal Xqi Xg must be located on the short arm of X chromosome
257	—				?
51	45 X 46 XX			−	?
101	—	−		−	
230	—	−		−	
44	—				?
136	45 X 46 XXr		−	−	Pat rnal Xr ?
91	—		−	−	Paternal Xr ?
130	45 X/46 XXq			−	?
100	45 X 46 XXqi			−	?
234	−	−			Maternal X and paternal Xqi Xg must be located on the short arm of X chromosome
160	45 X 46 XY	−		−	?
292	46 XX				

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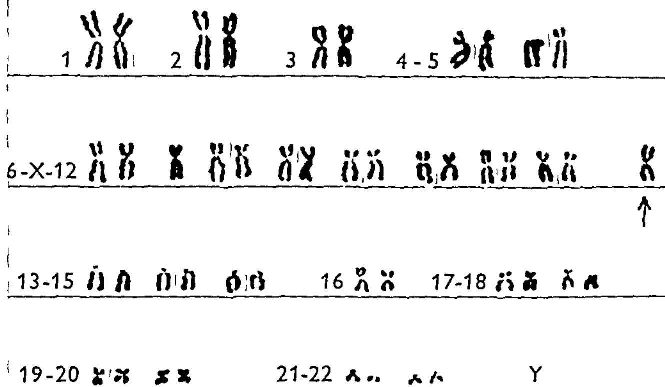


Fig 1 Pati nt 257 with i o hromo ome for the long arm of an X chromosome

sumed that she lacked the long arm of an X chromosome

Patient 292 with the karyotype 46 XX was sex chromatin positive With the present tech nique one cannot exclude that a minor un identifiable abnormality of one X chromo some may be present

Comparing Lindsten s material (4) of 57 patients with ours it can be seen that in both groups 50% 45 X constitution and 30–40% mosaicism were found In both series 1 pa tient with normal female karyotype and 2 pa tients with 46 XXqi (isochromosome for the long arm of an X chromosome) were present In Lindsten s material proportionally more 45 X/46 XXqi karyotypes were found whereas the present material had more 45 X/46 XXr (ring chromosome) Only in our series was a patient with 45 X/46 XY present No major differences could be found in the chromosomal patterns in patients with Turner s syndrome diagnosed either before or after the onset of normal puberty

In our material all patients with 45 X con stitution were sex chromatin negative whereas most patients with mosaicism 45 X/46 XX had sex chromatin in a lower number than in nor mal females 4 patients with mosaicism 45 X/ 46 XXr or 45 X/46 XXq were sex chromatin negative like the 45 X Both patients with 46 XXqi were sex chromatin positive In pa tients with 45 X/46 XXqi one could expect sex chromatin in a lower number than in nor mal females This was also the case in one patient (100) In the other patient (234) was found sex chromatin negative distribution This could possibly be due to the fact that only every fifth cell contained the isochromosome

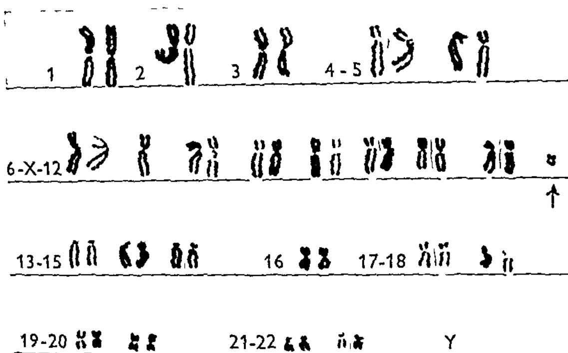


Fig 2 Patient 136 with ring chromosomes

whereas the rest of the cells only contained *one* X chromosome

From the above mentioned data it may be concluded that sex chromatin determination is not sufficient as a screening test for X chromosome abnormality in a patient with Turner's syndrome In the present material of 30 patients 4 were sex chromatin positive and 5 had sex chromatin in a lower number than in normal females In patients with sex chromatin positive distribution *one can* expect normal female karyotype or an isochromosome for the long arm of one of the X chromosomes and in patients with sex chromatin in a lower number than in normal females *one can* expect mosaicism of the karyotype

In 1962 Mann et al (6) described the Xg blood group system They found that the gene must be located on the X chromosome and that the gene determining the positive reaction must be dominant The gene was called Xg^a the silent gene for Xg and the antibody anti Xg^a Individuals reacting against the antibody were named Xg(+) and those not reacting Xg(−) While the gene is located on the X chromosome there is a possibility to determine the origin of the X chromosome in patients with X chromosome abnormality

In 1963 Lindsten et al (5) tested 56 father/mother/45 X daughter combinations for Xg blood group and found that in 20 cases the single X was of maternal origin and in one case of paternal origin The single X was found in our material in 2 patients (223 249) with a 45 X constitution of maternal origin

In 1963 Lindsten (4) reported a case of Turner's syndrome with the karyotype 46 XXqi and concluded that the isochromosome was *of paternal* origin and that the gene must be carried on the short arm of the X chromo-

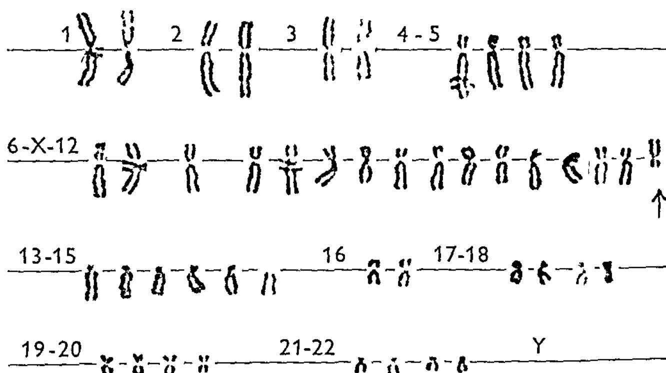


Fig 3 Patient 130 with deletion of the long arm of an X chromosome

some Our patients numbers 135 and 234 correspond in regard to karyotype and Xg blood group to Lindsten s findings mentioned

According to Lyon s theory one of the two X chromosomes in each somatic cell of the female is inactivated but it is a question whether the inactivation is complete and whether the Xg locus becomes inactivated It is known (4) that it is the isochromosome (or the deleted chromosome) which becomes inactivated In the above mentioned cases of patients with isochromosomes in both Lindsten s and our material the isochromosomes must be of paternal origin whether the Xg locus is inactivated or not (one may here conclude that the mothers must be heterozygotes for the Xg gene) But if the Xg locus is not inactivated one may conclude that the Xg locus must be located on the short arm of the X chromosome

Ring chromosomes are supposed to be formed by deletion of the arms and fusion of the ends of the arms There is some evidence that the Xg locus is located on the tip of the short arm (9) If this holds good then we may expect in patients 136 and 291 that the Xg locus has disappeared from the ring chromosomes and if so the ring chromosomes must be of paternal origin If the Xg locus has not disappeared from the ring chromosome and if the Xg locus is not inactivated then the ring chromosome may be of maternal origin

Objective measurements of the size of the sex chromatin has not been performed

SUMMARY

Determinations of chromosomal pattern sex chromatin and blood group determinations have been carried out in 30 patients with Tur

ner's syndrome in whom the diagnosis was made in childhood 15 patients had the karyotype 45 X 2 patients had 46 XXqi one had normal female karyotype and 12 patients had mosaicism in which one cell line was always 45 X and the other cell line either normal female or male karyotype or abnormality of one X chromosome Xg blood group determinations showed that in 4 of the 23 cases one could draw the conclusion that the missing X or the abnormal X must be of paternal origin Sex chromatin determinations showed that 21 patients were sex chromatin negative 4 were sex chromatin positive and 5 patients had sex chromatin in a lower number than in normal females

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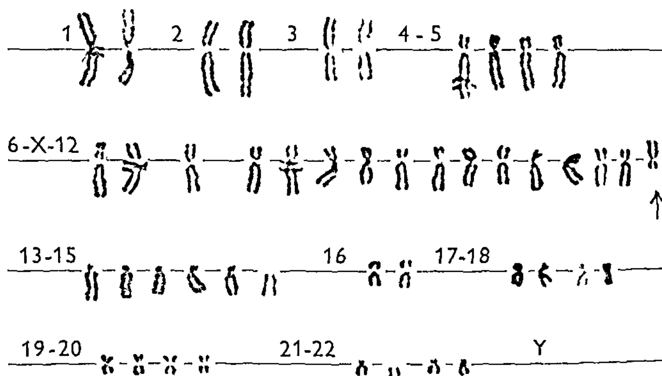


Fig 3 Patient 130 with deletion of the long arm of an X chromosome

some Our patients numbers 135 and 234 correspond in regard to karyotype and Xg blood group to Lindsten s findings mentioned

According to Lyon s theory one of the two X chromosomes in each somatic cell of the female is inactivated but it is a question whether the inactivation is complete and whether the Xg locus becomes inactivated It is known (4) that it is the isochromosome (or the deleted chromosome) which becomes inactivated In the above mentioned cases of patients with isochromosomes in both Lindsten s and our material the isochromosomes must be of paternal origin whether the Xg locus is inactivated or not (one may here conclude that the mothers must be heterozygotes for the Xg gene) But if the Xg locus is not inactivated one may conclude that the Xg locus must be located on the short arm of the X chromosome

Ring chromosomes are supposed to be formed by deletion of the arms and fusion of the ends of the arms There is some evidence that the Xg locus is located on the tip of the short arm (9) If this holds good then we may expect in patients 136 and 291 that the Xg locus has disappeared from the ring chromosomes and if so the ring chromosomes must be of paternal origin If the Xg locus has not disappeared from the ring chromosome and if the Xg locus is not inactivated then the ring chromosome may be of maternal origin

Objective measurements of the size of the sex chromatin has not been performed

SUMMARY

Determinations of chromosomal pattern sex chromatin and blood group determinations have been carried out in 30 patients with Tur

ner s syndrome in whom the diagnosis was made in childhood 15 patients had the karyo type 45 X 2 patients had 46 XXqi one had normal female karyotype and 12 patients had mosaicism in which one cell line was always 45 X and the other cell line either normal fe male or male karyotype or abnormality of one X chromosome Xg blood group determina tions showed that in 4 of the 23 cases one could draw the conclusion that the missing X or the abnormal X must be of paternal origin Sex chromatin determinations showed that 21 patients were sex chromatin negative 4 were sex chromatin positive and 5 patients had sex chromatin in a lower number than in normal females

ACKNOWLEDGEMENT

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STUDIES ON MATURITY IN NEWBORN INFANTS

II *External Characteristics*

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Premature infants (birth weight ⩽ 2 500 g) have long been regarded as having certain typical external characteristics such as abundant lanugo hair sparse subcutaneous fat short nails etc Since prematurity was defined by birth weight these signs could be regarded as typical of infants low in weight or small in size In the last decade it has often been pointed out that premature infants are a very heterogeneous group with respect to gestational age With this knowledge has followed a tendency to regard the old premature signs' as unreliable criteria of the gestational age of the infant and to ignore them in clinical practice However in recent years Farr et al (4, 5 6 7) have developed a method for maturity assessment (i e estimation of the gestational age) based on external characteristics of the infants A simple method for maturity assessment based on external characteristics has also been described by Usher et al (16) Both authors found the external characteristics to be of value in estimating gestational age of the newborn infant

Aims of the Present Study

1 To describe a simple technique for maturity studies in newborn infants based on external characteristics and to further evaluate external characteristics as maturity signs
2 To evaluate this method statistically and to present confidence limits for estimating gestational age with the aid of external characteristics
3 To study external characteristics in small for-gestational age and dysmature infants

Definitions and Abbreviations Used

Gestational age age in days from the first day of the mother s last menstrual period until the day of birth
Postmenstrual age age in days from the first day of the mother s last menstrual period until the day of examination
SGA small for gestational age infant i e birth weight below normal for gestational age (below −2 S D in the relation between birth weight and gestational age) according to Swedish standard curves (2 15)
AGA appropriate for gestational age infant Birth weight within normal limits for the gestational age
LGA large for gestational age infant Birth weight above normal for gestational age (above the *90th* percentile in this study)
Pre term gestational age less than 267 days post menstrual
Term gestational age 267 to 294 days
Post term gestational age more than 294 days
40th week days 274 to 280
Dysmaturity external signs of dysmaturity as described by Sjostedt et al (14) (stage I or more)

MATERIALS

The *investigated material* consisted of 174 newborn infants of various gestational ages For details about the material and its selection e Finnstrom 1971 (8)

Only infants for whom reliable information as to gestational age was obtained were included in the material Not all of these infants could be examined without the author s knowing their gestational age

A second material of 28 infants was examined after the investigated material This material served as a control of the prediction model constructed from the investigated material The age distribution was 225–238 days 2 infants 239–252 days 1 infant 253–266

days 5 infants 267–280 days 8 infants 281–294 days 10 infants and 295–308 days 2 infants The criteria for selection were the same as for th investi gated material The author did not know the gesta tional age of these infants at the time of examina tion

METHODS AND CRITERIA USED

Most of the infants in the inv stigated material were examined on the first or second day of life 3 in fants on the third day 5 on the 4th day and 1 on the 6th day They were examined undressed in good light The infants in the second mat rial were all ex mined on the first or second day A total of 12 external characteristics were scored Infants remain ing in the hospital more than 1 we k were examined once a we k until discharge Before the start of the study the author conducted a *pilot study* on 0 in fants in order to familiarize himself with the t chni que involved. Each characteristic was scored accord ing to 2 to 4 alternati e responses (scores) supposed to repre ent different degrees of maturation the highest number-d response corresponding to the highest degree of maturity The criteria used ar described below with their respective alternatives and scores and with references to earlier descriptions wh n these exist.

Brea.t si e Earlier u ed for maturity tudies by Keitel & Chu (12) Usher et al (16) and Farr et al (5) The transverse diameter is measured bilaterally with a sliding caliper and the largest value registered

1 Be ow 5 mm
2 5 to 10 mm
3 More than 10 mm

Nipple formation The characteristic used as describ d by Farr et al (4) Estimated by insp ction

1 Nipple barely visible no areola
  Nipple well-defined areola present but not raised
3 Nipple we l-defined edg of the areola raised above the skin

Skin opac ty The characteristic used essentially as described by Farr et al (4) Estimated by inspection of the trunk

1 Num rous veins tributari s and venules are clearly een particularly over the abdomen
  Veins and tributaries are seen
3 A few large blood ves els are clearly seen over th abdomen
4 A few large b ood ves els are indistinctly seen over the abdom n or no blood ves els are seen

Scalp hair The characteristic used as defined by Ush r et al (16) The scalp hair is inspected

1 Hair fine woolly or fuzzy Individual strands dif ficult to d stinguish
2 Hair coarse and silky Each hair appears as a single strand

Hair-forehead border The border is indistinct in low birth weight infants according to Kandler (13) The border is inspected

1 Border indistinct
2 Border distinct

Eyebrows The eyebrows are incompletely developed in low birth weight infants according to Kandler (13) Eyebrows inspected

1 Eyebrows are not seen
2 Eyebrows are incompletely developed (lateral part absent)
3 Eyebrows completely developed

Ear cartilage The characteristic used here es entially as described by von Harnack & Oster (11) The ears are palpated in order to estimate the distribution of ear cartilage In case there is a difference between the two ears the judgement is based on the most mature" ear

1 No cartilage is felt in antitragus
2 Cartilage is felt in antitragus
3 Cartilage is present in anthelix
4 Cartilage formation is completed in helix (i e cartilage can be palpated in the dorsal-cranial part)

Fingernails The characteristic used here essentially as described by von Harnack & Oster (11) The finger nails are inspected and the finger tip palpated (let the nail scratch the hand of the examiner)

1 The nails do not reach the finger tips
2 The nails reach the finger tips
3 The nails reach or pass the fingertips distal edge of the nail is distinct and relatively firm (i e the ed e of the nail can easily be felt if the nail scratches the hand of the investigator)

Xiphoid process The characteristic used essentially as described by Kandler (13) The xiphoid process is palpated and the way the process b nds is recorded

1 Xiphoid process is spoon formed i e turns up wards (ventrally)
2 Xiphoid process is in the same plane as the body of the sternum
3 Xiphoid process turns downwards (dorsally)

External genital organs

Boys degree of testicular descent Used both by Farr et al (5) and Usher et al (16) with slightly different alternatives The testes are inspected and palpated and the value from the right side is recorded

1 Testis incomp etely descended i e does not rea h the bottom of the scrotum
2 Testis completely descended i e reaches bottom of the scrotum

Girls female external genital organs Earlier us d by Farr et al (5) and older authors The genital organs are inspected

1 Labia majora do not completely cover labia mi nora
2 Labia majora completely cover labia minora or almost complet ly cover

Table 1 *Eight external characteristics analysed in frequency tables*

Infants were divided into gestational age groups of 2 week intervals The percentage distributions of separate respons alternatives for the different age groups are indicated in the table

Gestational age at birth (days)	Breast size			Nipple formation			Skin opacity				Scalp hair		Ear cartilage				Finger nails			Plantar skin creases				Pupillary membrane			
	1	2	3	1	2	3	1	2	3	4	1	2	1	2	3	4	1	2	3	1	2	3	4	1	2	3	
225	100	0	0	83	17	0	83	17	0	0	67	33	33	0	50	17	0	67	33	0	67	33	0	80	20	0	
225-238	50	50	0	10	90	0	50	40	0	10	70	30	0	40	50	10	10	30	60	0	30	60	10	20	30	50	1
239-252	11	89	0	6	55	39	22	72	6	0	39	61	0	17	61	22	0	28	72	0	6	72	22	6	35	59	1
253 266	14	83	3	0	24	76	10	52	17	21	17	83	0	3	38	59	0	31	69	0	3	59	38	0	28	72	2
267-280	2	83	15	0	13	87	12	25	46	17	5	95	0	12	46	42	0	5	95	0	0	12	88	0	34	66	4
281-294	2	59	39	0	8	92	6	24	43	27	2	98	0	6	31	63	0	6	94	0	0	18	82	2	17	81	5
294	0	44	56	0	0	100	0	22	50	28	0	100	0	6	22	72	0	0	100	0	0	0	100	0	6	94	1

Plantar skin creases The characteristic used here essentially as defined by Usher et al (16) Farr et al (4) give slightly different alternatives The sole of the foot is inspected Only the relatively broad creases are analysed Fine superficial lines may be present especially if the skin is dry but usually disappear if the sole is stretched from toes to heel

1 No skin creases are present
2 Anterior transverse creases only are present
3 Occasional creases are seen on the anterior two thirds of the sole
4 The whole sole is covered with creases i e also the heel

Pupillary membrane Although not actually an external characteristic this was included It has long been known that pre term infants can have remains of the pupillary membrane which was used by von Harnack & Oster in their maturity scoring (11) The pupillary plane is examined after widening of the pupils with cyclopentholate (Cyclogyl®) with the aid of an ophthalmoscope

1 Membrane rests are seen in the form of distinct arcades
2 One or several separate strands are seen uni or bilaterally
3 No membrane rests are seen over the widened pupil

Statistical methods

See Finnstrom 1971 (8)

RESULTS

The 12 external characteristics were analysed in frequency tables giving the absolute and per centage distributions of the response alterna tives within different gestational age groups The result of this analysis with respect to the eight characteristics used in the final scoring (see below) is shown in Table 1 the infants being divided into gestational age groups of 2 week intervals For all eight characteristics there was a rather smooth change from low (immature) to high (mature) alternatives with advancing gestational age

The *external characteristics* studied were *individually correlated to gestational age* the degree of correlation varied from 0 12 to 0 68 See Table 2 External genital organs scored higher for boys than for girls at the same gestational age

In analysing the external characteristics in *stepwise multiple regression* with age as dependent variable five characteristics entered the multiple regression at significant ($p < 0\ 05$) F values giving a multiple correlation coefficien

Table 2 *Coefficients for the correlations between individual external characteristics and gestational age*

The multiple correlation coefficient was 0 84

External characteristic	Coefficient of correlation
Nipple formation	0 68
Plantar skin creases	0 65
Breast size	0 62
Scalp hair	0 51
Skin opacity	0 48
Pupillary membrane	0 45
Genital organs	0 43
Fingernails	0 41
Ear cartilage	0 41
Hair-forehead border	0 17
Eyebrows	0 15
Xiphoid process	0 12

Table 3 *Means and S.D.s for total maturity scores (external characteristics)*

Infants divided into gestational age groups of two week intervals. Minimal possible score 0.31, maximal possible score 1.00

	Gestational age at birth, days						
	<225	225–238	239–252	253–266	267–280	281–294	>294
Total maturity score							
Mean	0.50	0.64	0.74	0.82	0.86	0.90	0.93
S.D.	0.11	0.04	0.05	0.06	0.07	0.07	0.04
No. of infants	6	10	18	29	42	51	18

of 0.84. These characteristics were nipple formation, plantar skin creases, breast size, scalp hair and ear cartilage.

Maturity scoring. The scores for the individual characteristics were summed to a total maturity score, as suggested by Farr et al. (5). Several models for summing and selecting the characteristics were tried.

The score found to be best was constructed as follows: the individual scores for eight characteristics (those that showed the highest individual correlation to gestational age, excluding external genital organs, see above) were summed and this sum was divided by the maximal maturity score possible for the individual infant, thus making it possible to use the *score* even if the value for one characteristic was missing.

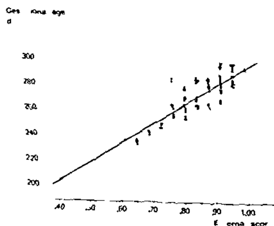


Fig. 1. Total maturity score based *on eight* external characteristics correlated to gestational age at birth for 174 newborn infants. Linear regression line indicated in the figure.

The *means and standard deviations of the total maturity scores* are shown in Table 3, the infants being divided into gestational age groups of two week intervals.

The total maturity score was *correlated to gestational age* (see Fig. 1) with Y (gestational age) as dependent variable of X (maturity score). The linear regression equation was $Y = 145.62 + 149.38\ X$.

Compared with the values actually observed in the investigated material, this formula has a slight tendency to overestimate gestational age at low values of the score and a slight tendency to underestimate age at high values of the score. The correlation coefficient was 0.82. The logarithmic function, power function and the second degree function gave no better explanation of the relation between score and gestational age. The correlation coefficient (multiple r) for the logarithmic function was 0.82, for the power function 0.84, and the multiple r for the second degree function was 0.83.

The separate regression lines for *boys and girls* did not differ significantly ($p > 0.05$) with respect to slope and position.

The 95% *confidence limits* for estimating gestational age from different values of X were ± 24.3 days (mean score for infants *younger* than 225 days), ± 24.0 days (mean score for all infants) and ± 24.0 days (mean score for infants older than 294 days).

Multiple regression analysis gave a prediction interval of ± 22.7 days for mean values of the different criteria *entering the multiple* regression at significant F values.

The mean maturity score for 18 *small-for-gestational age infants* as compared with pre term and term AGA infants is shown in Table 4 The mean score for the full term AGA group was significantly higher than that for the SGA group of identical mean age The mean score for the SGA group was significantly higher than that for the pre-term group of identical mean birth weight A comparison of full term SGA and AGA infants with respect to individual characteristics showed that a difference existed for all characteristics except two, nipple formation and ear cartilage The difference was most pronounced for skin opacity The same comparison between SGA infants and pre term infants showed that only fingernails had the same value for the two groups For all other characteristics, the pre-term infants had lower values

The corresponding values for nine *large-for-gestational age* infants and their control infants of normal birth weight is shown in Table 5 There was no significant difference in mean score

Finally the maturity scores for *dysmature* infants are shown in Fig 2 in which the scores for dysmature and non dysmature infants are plotted against gestational age Dysmaturity was defined according to Sjostedt et al (14) thus only external signs of dysmaturity were taken into consideration The mean value of the score for infants with dysmaturity (only mild forms stages I and II according to Sjostedt et al (14) were seen) was 0 87 at a mean age of 282 1 days ($N = 52$) giving a slightly lower score than expected for this age

Repeated examinations of the external characteristics at 1 week intervals were performed on 93 occasions on 40 infants Infants with a postmenstrual age exceeding 308 days were not included in this study The scores for these examinations were correlated to postmenstrual age at examination the correlation coefficient being

Table 5 *Mean total maturity scores based on eight external characteristics mean gestational ages and mean birth weights of large for gestational age and appropriate for gestational age infants*

Asterisks indicate significant difference between mean values

	No of infants	Age at birth (days)	Birth weight (grams)	Maturity score
LGA	9	287	4 358 ***	0 91
AGA	9	285	3 307	0 90

*** $p<0\ 001$

Table 4 *Mean total maturity scores based on eight external characteristics mean gestational ages and mean birth weights of full term appropriate for gestational age full term small for gestational age and pre term appropriate for gestational age infants*

Asterisks indicate significant differences between mean values

	No of infants	Age at birth (days)	Birth weight (grams)	Total maturity score	
Full term SGA	18	276	2 133 ***	0 79 ***	
Full term AGA	18	276 ***	3 399 ***	0 88 ***	**
Pre term AGA	18	242	2 134	0 69	

** $p<0\ 01$ *** $p<0\ 001$

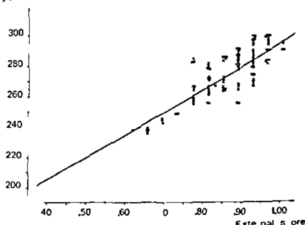


Fig 2 Maturity score correlated to gestational age at birth Y indicates infant with signs of dysmaturity ($N = 52$) Linear regression line indicated in the figure

0 32 Nineteen per cent of the repeated examinations were performed on SGA infants compared with 11% of the first examinations on all infants The regression equation for the repeated examinations was $Y = 206\,82 + 74\,16\,X$ giving a less steep regression line than the original one Compared with the original line the line for repeated examinations overestimates gestational age at low values of the score by 3 -5½ weeks It underestimates gestational age at high values of the score by 1-2 weeks

The *second material* of 28 infants was examined with the same technique in order to test the accuracy of the prediction model constructed earlier The gestational age of 1 infant was overestimated by 19 days The gestational age of 3 infants was underestimated by 21 26 and 26 days respectively The rest of the infants were correctly estimated within 17 days

The *reproducibility of the methods* was tested as follows 20 infants not included in the two materials described above were examined twice on the same day (morning and afternoon) The results of the first examination were not available at the second examination The total scores (without division) for the two examinations varied by 0-2 points A difference of 2 points corresponds to a gestational age difference of about 11 days

DISCUSSION

The 12 *external characteristics used* in this study were chosen mainly because they have been claimed to be of value in maturity assessments and because the author *found them easy to define and evaluate* There was rarely any difficulty in choosing the appropriate alternative (score) of those given in the definitions In order to minimize the possibility that the scoring might be influenced by his increasing experience during the course of the investigation the author conducted a pilot study before the start of the investigation performing repeated examinations on 20 infants

The scoring as done by the author was satisfactorily reproducible The agreement between different observers was not tested but has been reported to be high in a corresponding study (4)

Three of the characteristics studied hair forehead border eyebrows and xiphoid process showed very low correlations to gestational age as judged by the low individual correlation coefficients and are probably of no value in maturity assessments Of the remaining nine criteria three showed good correlation to gestational age nipple formation plantar skin creases and breast size The rest of the characteristics showed fairly good correlation to gestational age It is obvious that for the individual criteria studied there was a smooth change in distribution from low to high alternatives (scores) with advancing age This means that there is no definite age limit at which the majority of infants suddenly change from a lower to a higher score There are very few exceptions With respect to nipple formation there is a distinct change in distribution of the responses for infants below and above 225 days gestational age However it is impossible to estimate gestational age from the study of only one or a few external characteristics

Therefore it seems advisable to calculate a *total maturity score* by summing the scores for individual criteria as suggested by Farr et al (5) The total maturity score thus arrived at can then be used for calculations in the same way as results from studies in which a quantitative result is obtained (e g birth weight) The score can be constructed in different ways It was found that the score that showed the best correlation to gestational age was the one from which four of the original 12 criteria were excluded These were the three that showed very low individual correlations to gestational age and one which showed rather pronounced differences between boys and girls (external genital organs)

A linear *regression equation* was used for the relation between the maturity score and gestational age since other types of equations (logarithmic function power function and second degree function) did not significantly change

The mean maturity score for 18 *small for gestational age infants* was compared with pre-term and term AGA infants is shown in Table 4 The mean score for the full term AGA group was significantly higher than that for the SGA group of identical mean age The mean score for the SGA group was significantly higher than that for the pre term group of identical mean birth weight A comparison of full term SGA and AGA infants with respect to individual characteristics showed that a difference existed for all characteristics except two nipple formation and ear cartilage The difference was most pronounced for skin opacity The same comparison between SGA infants and pre term infants showed that only fingernails had the same value for the two groups For all other characteristics, the pre term infants had lower values

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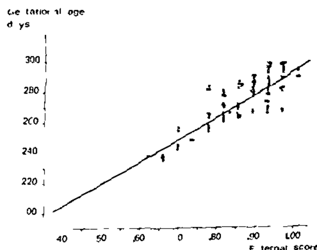


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Therefore it seems advisable to calculate a *total maturity score* by summing the scores for individual criteria as suggested by Farr et al (5) The total maturity score thus arrived at can then be used for calculations in the same way as results from studies in which a quantitative result is obtained (e g birth weight) The score can be constructed in different ways *It was found that* the score that showed the best *correlation* to gestational age was the one from which four of the original 12 criteria were excluded These were the three that showed very low individual correlations to gestational age and one which showed rather pronounced *differences between* boys and girls (external genital organs)

A linear *regression equation* was used for the relation between the maturity score and gestational age since other types of *equations* (logarithmic function power function and *second degree function*) did not significantly change

the degree of correlation The regression line can be used for both boys and girls since the separate regression lines for the two sexes did not differ significantly The slight tendency to underestimate gestational age at high values of the score and to overestimate age at low values of the score should be noted

In calculating the regression equation data from only one examination of each child was used although several of the infants had been examined more than once some as many as six times Using the results from the repeated examinations would increase the risk of errors in the formula If for example a mother's information about the gestational age were erroneous in spite of all precautions this error could be of importance if the results of several examinations on this child were included Furthermore when the calculations were done it was not known whether a maturity assessment based on external characteristics is reliable even when made after the first week of life see below The author therefore believes it is incorrect to use the results of more than one examination of each child for the calculation of the regression equation of Dubowitz et al (1)

The *prediction interval* (95 % confidence limits) for different values of the maturity score varied from ±24 3 to ±24 0 days The 95% *confidence limits based on birth weight* were ±28 9 days (8) The prediction interval presented in this study is considerably broader than that presented by Farr et al (5) and Dubowitz et al (1) In both cases the intervals were claimed to be ±2 4 weeks (i e 17 days) probably for mean values of the score It should be pointed out that the intervals given by Farr et al and Dubowitz et al are probably valid only for mean values of the score for which the narrowest confidence limit is to be found In the present study the confidence limits were of the same magnitude whether derived from the mean or from more extreme values of the score However the main reason that the intervals found by Farr et al and Dubowitz et al differ from those found by the author is probably that there were differences in age distribution in the materials and differences regarding the proportion of SGA infants (10)

It is important to note that Farr et al (5) found a surprisingly good estimation from birth weight alone ±3 0 weeks (±21 days) a prediction interval only 4 days broader than that obtained from the maturity score (±5 days difference in the authors study) A method for maturity assessment can hardly be evaluated unless it is compared with some sort of reference method e g calculation from birth weight If this is done, the methods presented by Farr et al (5) and by the author are quite comparable *Dubowitz et al (1) did not state* the prediction interval obtained from birth weight Ideally the prediction model presented should always be tested on a new material not included in construction of the formula as in the present study

For further discussion of the difficulties in evaluating prediction intervals see Finnstrom 1972 (10) External characteristics and neurological signs were the best of the criteria for maturity assessment studied by the author (10) They were better than anthropometric data postnatal radiological examination of epiphyseal centers or motor conduction velocity Scoring of external characteristics has some advantage over neurological tests since the examination *is easier to perform and independent of the* state of the infant and can be performed immediately after birth

The prediction interval obtained by analysing several external characteristics in multiple regression gave only a slightly narrower limit Thus the complicated multiple regression analysis has very limited advantages over the simple scoring system as already pointed out by Farr et al (5)

The finding of significantly lower scores for *small for gestational age infants* than for AGA infants of identical mean gestational age is in accord with the findings of Farr & Mitchell (6) who showed that birth weight had an effect on maturity score since at a given gestational age the score increased with increasing birth weight The difference in mean score between the SGA

group and the AGA group in the present material corresponded to the rather great difference in age of 13 days On the other hand the score for the SGA infants was significantly higher than that for the pre term infants of identical mean birth weight Since only two of the characteristics used in the score showed the same value for SGA as for AGA infants it will probably be difficult to find a scoring system which does not underestimate gestational age in SGA infants It was a little surprising that ear cartilage was one of the characteristics unaffected by intra uterine growth retardation since it is known that epiphyseal development is retarded in these infants (9) The pronounced difference in skin opacity found between the two groups probably depends on the relative lack of subcutaneous fat in the SGA infants Measurement of subcutaneous fat was not included in the present study since it has been shown that this measurement is of no value in maturity assessment (3)

The tendency towards lower scores than expected in *dysmature* infants was not analysed statistically since these infants could not be compared with other infants of the same mean age Reduced subcutaneous fat which is one typical feature in these infants probably explains a large part of the noted difference

The results of the *repeated studies* on some of the infants indicate that maturity assessment based on external characteristics must be evaluated with caution after the first week of age

Practical use of the external scoring system

The following external characteristics are evaluated breast size nipple formation skin opacity scalp hair ear cartilage fingernails plantar skin creases and pupillary membrane The scoring system can be used even if the result for one of the characteristics is lacking It is advisable to score all eight characteristics however The scores for the individual characteristics are summed this sum is divided by the maximal score possible for the infant i e 26 if all characteristics are scored The final figure arrived at can either be used in the regression equation given or used for extrapolating gestational age from the regression line in Fig 1

SUMMARY

A simple technique for estimating maturity in the newborn period based on eight external characteristics is described

A maturity score based on these characteristics was shown to correlate well to gestational age and to give a fair estimate of the gestational age of newborn infants A formula for estimating gestational age based on the study of 174 infants is presented

The prediction interval for estimating gestational age on the basis of the mean value of the maturity score was ±24 0 days The formula was tested with a second material of 28 infants A correct estimation of gestational age within 17 days was possible for 24 of these infants

Small for gestational age infants had significantly lower mean maturity scores than appropriate for gestational age infants of the same mean gestational age They had higher scores than pre term appropriate for gestational age infants of the same birth weight Dysmature infants (external signs of dysmaturity) probably have lower scores than expected for their gestational age

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STUDIES ON MATURITY IN NEWBORN INFANTS

VI Comparison between Different Methods for Maturity Estimation

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Estimation of maturity and thereby gestational age in newborn infants is of value from several aspects It is of practical importance for the correct management of the individual infant since clinical problems depend both on the gestational age of an infant and on the birth weight and reliable information as to the gestational age is not always available Secondly materials on which neonatal physiological studies are to be carried out must be controlled with respect to gestational age (7) Thirdly knowledge of the true gestational age is important in developmental testing of preterm infants (25)

Since birth weight is now known to be an unreliable index of maturity and gestational age there has been increasing interest during recent years in finding and testing new methods for estimating gestational age in newborn infants These methods have recently been extensively reviewed (7)

For various reasons to be discussed below it is difficult to evaluate the results of most of these studies Certainly there has been a tendency in much of this work to overestimate the precision of estimating gestational age

There is a need for studies in which different methods for estimating maturity are applied to the same material of newborn infants making it easier to compare different methods Such a comparison is made in the present study which is based in part on five earlier studies on maturity in 174 newborn infants (4 15 16 17 18)

Aims of the Present Study

1 To compare five different methods for estimating maturity in newborn infants as well as combinations of two or more of these methods
2 To investigate the sources of error in estimating gestational age
3 To evaluate the methods tested by the author and to compare them with those described in the literature

Definitions and Abbreviations Used

Gestational age age in days from the first day of the mother s last menstrual period until the day of birth

Postmenstrual age age in days from the first day of the mother s last menstrual period until the day of examination

SGA small for gestational age infant Birth weight below −2 SD in the relation between birth weight and gestational age according to Swedish standard curves (12 32)

AGA appropriate for gestational age infant Birth weight within normal limits for the gestational age

LGA large for gestational age infant Birth weight above the 90th percentile in the relation between birth weight and gestational age

Pre term gestational age less than 267 days post menstrual

Term gestational age 267 to 294 days

Table 1 *The 95% confidence limits for estimating gestational age from mean values of Y or combinations of Y*

Variable or combination of variables	Confidence limits (± days)
Bi parietal diameter	34 7
MCV ulnar nerve	32 9
Crown-heel length	29 7
Distal femoral epiphysis	29 3
Birth weight	28 9
Birth weight + crown-heel length	28 6
Femoral + calcaneus epiphyses	27 5
Head circumference	26 1
Neurological examination	24 3
External characteristics	24 0
External characteristics + head circumference	22 4
External characteristics + neurological examination	21 0
External characteristics + neurological examination + head circumference	20 4
External characteristics + neurological examination + MCV ulnar nerve	20 1
External characteristics + neurological examination + head circumference + MCV ulnar nerve	19 7
External characteristics + neurological examination + head circumference + femoral epiphysis + MCV ulnar nerve	19 5

Post term gestational age more than 294 days post menstrual
40th week days 274–280
LMP last menstrual period
MCV motor conduction velocity

MATERIALS

The *investigated material* consisted of 174 newborn infants of various gestational ages Only infants for whom reliable information as to gestational age was obtained were included in the material Details about the material and its selection have been given earlier (15)

A second material of 28 infants was examined after the investigated material and served as a control of the prediction models constructed from the investigated material For details about this material see previous papers (16 17) *The author did not know* the gestational age of these infants at the time of examination

METHODS

The following parameters were used in estimating the maturity of newborn infants anthropometric measurements (15) external characteristics (16) neurological tests (17) postnatal examination of epiphyseal centers (18) and *motor conduction velocity* (4) For detailed descriptions see the respective papers

Statistical methods are described in a previous paper (15)

RESULTS

I *Estimation of gestational age in the investigated material*

The mean values and standard deviations for different parameters the infants being divided into gestational age groups of 2 week intervals, have been given in earlier papers (4, 15, 16 17, 18) together with the correlation coefficients and the linear regression equations Some *of the linear regression equations for estimating* gestational age are given below as are also the multiple regression equations for estimating gestational age from combinations of methods and/or measurements For all variables studied the gestational age at birth has been used in calculating the equations

External characteristics
$Y = 145\ 62 + 149\ 38\ X_1$

Neurological tests
$Y = 140\ 70 + 153\ 30\ X_2$

Head circumference
$Y = 11\ 03 + 7\ 75\ X_3$

Distal femoral epiphysis
$Y = 245\ 06 + 5\ 87\ X_4$

MCV ulnar nerve
$Y = 191\ 42 + 2\ 99\ X$

For combinations of parameters
External characteristics and head circumference

$Y = 73\ 63 + 99\ 70\ X_1 + 3\ 39\ X_3$

External characteristics and neurological tests

$Y = 127\ 60 + 85\ 81\ Y_1 + 84\ 07\ X$

External characteristics neurological tests and head circumference

$Y = 83\ 29 + 63\ 02\ X_1 + 71\ 28\ X + 2\ 22\ X_3$

External characteristics neurological tests head circumference and MCV ulnar nerve

$Y = 85\ 16 + 55\ 67\ Y_1 + 66\ 48\ X + 1\ 87\ X_3 + 0\ 76\ X_5$

External characteristics neurological tests, head circumference femoral epiphyses and MCV ulnar nerve

$Y = 102\ 84 + 50\ 90\ X_1 + 58\ 08\ X\ + 1\ 51\ X_3 + 0\ 11\ X_4 + 0\ 78\ X_5$

where X_1 = external score
X = neurological score
X_3 = head circumference in cm
X_4 = femoral epiphysis largest diameter in mm
X_5 = MCV ulnar nerve in m/sec

The 95 / *confidence limits for estimating gestational age* from mean values of X or combinations of X are given in Table 1 For combinations of methods values are given only for those combinations in which the variables entered the multiple regression at significant ($p < 0\ 05$) F values Adding further variables did not significantly change the estimate As can be seen in the table it was possible to decrease the confidence limits by $\pm 9\ 4$ days by using a combination of five methods instead of birth weight

The *effect on the confidence limits* of changes in the investigated material are shown in Table 2 Thus changing the material by selecting either only infants with birth weight above the 10th percentile or only girls or boys led to pronounced changes of the SD of gestational age the values of the correlation coefficients and thereby also the breadth of the 95 confidence limits

Table 2 *Effect of changes in the material on the breadth of the confidence limits as exemplified by the values for head circumference*

Material	95 confidence limits ± days	SD age	Correlation coefficient	Number
All infants	6 1	1 2	0 78	173
Girls	4 5	17 0	0 70	85
Boys	239	240	0 87	88
Infants birth weight above the 10th percentile	707	19 3	0 84	119

Table 3 *Estimation of gestational ages in the second material (control of prediction models)* *N* 28

Variable used	Number of ages correctly estimated within ±14 days	±17 days	±21 days
Head circumference	21	22	25
External score	18	24	26
Neurological score	24	26	27
External score + head circumference	24	24	26
External score + neurological score	22	27	28
External score + neurological score + head circumference	24	27	28

II *Estimation of gestational age in the second material*

By using prediction models constructed from the investigated material the gestational ages of the infants in the second material were estimated These ages were compared with the gestational ages calculated from the mother s LMP See Table 3 In this material neurological tests estimated gestational age slightly better than did external characteristics By combining variables the number of correctly estimated ages could be increased Adding more variables than the three indicated in the table did not change the precision of estimation

III *Variation in time lag between the first day of the last menstrual period and the probable day of conception*

In order to investigate the error in estimating gestational age from data on LMP half of the mothers in the investigated material and all the mothers in the second material were questioned about the possible date of intercourse leading to pregnancy

25 mothers in the investigated material and 9 in the second material could state this date Most of the mothers gave an exact day the others named 2 or 3 successive possible days The mean intervals between LMP and intercourse were 13 2 and 11 0 days in the two materials giving a mean of 12 6 days for the

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External characteristics neurological tests head circumference and MCV ulnar nerve

$Y = 85\ 16 + 55\ 67\ X_1 + 66\ 48\ X\ + 1\ 87\ X_3 + 0\ 76\ X_5$

which the time of ovulation or the time of a *single intercourse leading to pregnancy* is known would increase the precision of estimation However studies of the infants whose mothers could state the day of intercourse leading to pregnancy indicated that the increase in precision is rather small A prediction model using a single method and based on LMP will probably not permit precision in the estimation of ±2 weeks or even less as has been claimed by several authors (8 9 23 28)

II *Comparison between the methods used by the author*

These methods and combinations of them can easily be compared since they were applied to the same material of newborn infants Some of the examinations were not performed immediately after birth but during the first week (neurological examinations radiological examinations and motor conduction velocity) Thus for the same infant different examinations were performed at different postmenstrual ages However in constructing prediction models using more than one method only one age could be used as the dependent variable in the stepwise regression analysis Therefore the gestational age at birth had to be used for all analyses omitting the postmenstrual ages at examination This could be done since it was shown (4 17 18) that this approximation did not lead to any important error

Th calculated 95 % confidence limits for estimating gestational age can be used for these comparisons Some findings deserve comment Calculating age from birth weight was used as a reference method It can be seen that biparietal diameter gives a considerably poorer estimate of gestational age than does birth weight Radiological measurements of epiphyseal centers even if measurements of several centers are combined do not give a substantially better estimation than birth weight Head circumference is definitely a better measurement than birth weight The two best single methods were obviously scoring of external characteristics and neurological tests decreasing the confidence limits by ±5 days compared with birth weight The precision of estimating gestational age was further increased by the use of any two or all of the following head circumference external characteristics and neurological tests This has earlier been shown for the combination of external characteristics and neurological tests (9) Adding further variables to the prediction model did not give any important increase in precision Thus a fair estimate of gestational age can be made from methods which do not require any technical facilities and which are relatively simple to perform although the examiner needs experience to perform and evaluate a neurological examination The results of the control of the prediction models using the second material indicate that it is possible to estimate gestational age to within two and a half weeks by combining methods See Table 3

For routine clinical use in the neonatal period the *scoring of external characteristics* perhaps in combination with measurement of head circumference seems to be the method of choice among those *studied by the author* If maturity assessment is indicated later than during the first week of life *neurological testing* seems to be more reliable than external *scoring (16 17)*

A disadvantage of most of the methods studied by the author is that they underestimate gestational age in small for gestational age infants (defined as birth weight below −2 S D) This is probably also true of other methods not used by the author The difference in opinion regarding the estimation of gestational age in SGA infants by e g neurological tests (17 19 28) might well depend on the criteria used for selecting SGA infants as has been discussed earlier (15) For further discussion of the reliability of standard curves for intrauterine growth see Tanner (34)

At present the only method that does not seem to underestimate gestational age in SGA infants is MCV (4 29) MCV the scatter in results at a given gestational age being wide is not very suitable for maturity estimation in

groups combined The intervals varied between 7 and 22 days, S D was 3 8 days

DISCUSSION

The following subjects will be discussed factors limiting the accuracy of models used for estimating gestational age comparison between methods used by the author and comparison with results from other studies

I *Factors limiting the accuracy of models used for estimating gestational age*

Errors due to the use of LMP in calculating gestational age

Most studies on maturity in newborn infants are based on materials in which gestational age was calculated from LMP This involves some possible errors First the date for LMP may be erroneous It is therefore necessary to check these dates very carefully as has been discussed earlier (15) Secondly there is variation in the time lag from LMP to ovulation Conception is possible throughout the whole cycle (21) There can also be a time lag between the time of intercourse leading to pregnancy and ovulation or between ovulation and intercourse of ±1–2 days In the present material the time lag between LMP and intercourse leading to pregnancy was 12 6 days S D 3 8 If this sample is representative of a randomly selected normally distributed sample this means that the interval would be within 12 6±7 6 days for 95% of women Therefore it is reasonable to believe that the error in estimating gestational age from LMP amounts to at least ±1 week even if the dates for LMP are carefully verified

Limitations in the reproducibility of the methods

A variation of 1 cm in measuring head circumference or 2 m/sec in recording MCV corresponds to a variation in gestational age of about 5 days (in the present study) A variation of 2 points when scoring external characteristics or neurological tests corresponds to a variation in gestational age of about 11 and 8 days respectively These figures are based on studies of the reproducibility of the methods described earlier (4 16 17) It is not likely that other methods used for maturity estimations have a much higher degree of reproducibility than those used by the author Little is known about the agreement between different observers It has been reported to be high for external characteristics (9 13) and neurological tests (9) but it was not stated how close the correspondence was between estimates of gestational age by different observers More information about the agreement between different observers would be of value for all methods used for maturity studies

When two or more methods are combined the errors due to lack of reproducibility are reduced and this is one reason why the confidence limits decrease when a combination of two or more methods is used

Biological variation

This is the third factor limiting the accuracy of prediction models It has been discussed earlier in connection with MCV (4) There is every reason to believe that there is true variation in development among infants of the same gestational age Studies on MCV and neurological development in infants whose mothers could state the probable day of conception supports this (Blom & Finnstrom unpublished) The extent of this variation is not known but examination of the 34 infants whose probable date of conception was known suggested that it corresponds to at least ±1 week

When the above three sources of error are taken into consideration it can be stated that prediction models (single methods) which can be empirically shown to estimate gestational age to within 3 weeks from that calculated from LMP for all but a few infants can be regarded as satisfactory This is especially true if this degree of precision is also obtained for infants of low or high gestational age Basing the prediction model on a material of infants for

They did not correct for birth weight however SGA infants probably have an increased rate of fetal hemoglobin synthesis (1) A disadvantage of measuring fetal hemoglobin is that the procedure is rather complicated and time-consuming The value of the estimation of fetal hemoglobin in assessing the maturity of new born infants needs to be further studied

As to other variables used or recommended for maturity studies EEG sleep studies and evoked potentials have been discussed elsewhere (17) Changes in the fundus of the eye have been reported to appear with advancing gestational age (24) The value of these changes for maturity assessment is not clear The same is true of reflex arc latency which recently has been shown to increase with advancing gestational age (10) Chemical analyses have shown changes related to gestational age for enzymes in the meconium (11) and serum enzymes (GPT) (22) Oxygen consumption studies have shown differences between pre term SGA and full term AGA infants (31) The level of immunoglobulin G increases with advancing age (2 33 35) SGA infants have decreased levels as compared with AGA infants of the same age (26 35) Total red cell volume increases with gestational age and the quotient total red cell volume/birth weight shows a slight decrease with advancing gestational age (5) For all these biochemical parameters the variation at a given gestational age is too great for them to be of any practical value in maturity assessment. For further review of methods for maturity assessment see Casaer & Akiyama (7)

At present the following conclusions apply The *best methods for postnatal maturity assessment* are external characteristics or neurological tests see also Casaer & Akiyama (7) von Harnack & von Bernuth (20) Head circumference is also a valuable measurement The use of combinations of these methods increases the precision of estimating gestational age EEG sleep studies is an interesting method but it is time-consuming and needs to be further evaluated Further studies of fetal hemoglobin would also be of interest

SUMMARY

Five different methods for maturity assessment were applied to a material of 174 newborn infants of various gestational ages

The methods consisted of measuring or scoring the following parameters anthropometric measurements external characteristics, neurological tests examination of epiphyseal centers and motor conduction velocity

Prediction models for estimating gestational age using single methods or combinations of methods are given

The best single methods were scoring of external characteristics and neurological tests the former being the most suitable for routine work during the first postnatal week

All methods except motor conduction velocity underestimate gestational age in small for gestational age infants

By combining methods the precision of estimating gestational age increases Useful combinations are any two or all of the following external characteristics neurological tests and head circumference

The precision of the prediction models was tested using a second material of 28 infants not included in the construction of the models The results of these tests indicate that it is possible to obtain a precision in the age estimation of $\pm 2^1/_2$ weeks for about 95% of the infants (27 of 28 in this study) using a combination of methods

Factors limiting the precision of estimating gestational age are the time variation between LMP and conception the degree of reproducibility of the methods and the biological variation in maturity at a given gestational age

Various difficulties in evaluating the results of maturity studies are discussed It is pointed out that new methods must be compared with a simple and precise reference method based for example on birth weight applied to the same material Comparison between different methods applied to different materials must be made with caution and the commonly used 95% confidence limits are not suitable for such comparison

individual newborn infants Since MCV seems to be unaffected in different pathological conditions (4, 30) it is a suitable method to use when the gestational age of groups of newborn infants is to be controlled (e g for neonatal physiological studies)

III *Comparison with results from other studies*

It is difficult to compare the results of the present study with those of other studies reported in the literature Saint-Anne Dargassies (27, 28) whose work on neurological maturity and maturation in newborn infants has been of great importance give no statistical evalua tion of her results In some papers (9 23) comparison with other reports has been based on comparisons of the calculated 95% con fidence limits for estimating gestational age This type of comparison is not valid The breadth of the confidence limits depend not only on the values of the correlation coeffi cients (for the correlation between age and values of the investigated parameter) but also on the age distribution (S D of age) in the material studied This is obvious from the example given in Table 2 as well as from the prediction formula used (15) Therefore con fidence limits calculated from studies on one material cannot be compared with confidence limits from another material unless the age distributions in the two materials are identical If the correlation coefficient remains constant although the standard deviation for gestational age increases the breadth of the confidence limits increases as well

The confidence limits have also been wrongly interpreted in another context Some authors (6, 23) claim to have reached the upper possible limit of precision in estimating gestational age since the 95% confidence limits for estim ating age (from mean value of fetal hemoglobin) is close to the 95% confidence limits for the duration of pregnancy per se These two confidence limits refer to different phen omena however and cannot be compared One reflects the precision of a method for estimat ing gestational age, the other the natural range for the duration of pregnancy

However it is possible to make a comparison based on confidence limits between two reports on maturity assessment if both reports give con fidence limits not only for one investigated method (e g external characteristics) but also for a common objective and precise *reference method* (e g calculating age from birth weight) The differences in confidence limits between the reference method and the investigated meth od in the two reports can then be compared regardless of the actual breadths of the con fidence limits It would be of great value for future comparisons and evaluations if this type of reference method was always included Un fortunately the confidence limits for birth weight have been given in only a few papers (6 14) For the hitherto published materials the confidence limits have been given only for mean values of the variable studied In evaluat ing the precision of estimating gestational age it must be known whether the prediction model is also applicable at high and low values of the variable (thus corresponding to high and low gestational ages) and the confidence limits should be calculated for these extreme values as well

Farr et al (14) compared external charac teristics with birth weight and found that the former method decreased the confidence limits by ±4 days and Dubowitz et al (9) compared external characteristics with neurological tests and found the confidence limits to be the same Brody (6) found that the use of the quotient percent of fetal hemoglobin birth weight gave a remarkably better estimation of gestational age than the use of birth weight (±9 5 days difference) Kirschbaum (23) using column chromatography for determining fetal hemoglobin gave very narrow 95% confidence limits for estimating gestational age from the percentage of fetal hemoglobin (±13 9 days) Confidence limits for estimating age from birth weight were not given Later authors (1 3) pointed out the wide scatter in the percentage of fetal hemoglobin at a given gestational age

They did not correct for birth weight however SGA infants probably have an increased rate of fetal hemoglobin synthesis (1) A disadvantage of measuring fetal hemoglobin is that the procedure is rather complicated and time consuming The value of the estimation of fetal hemoglobin in assessing the maturity of newborn infants needs to be further studied

As to other variables used or recommended for maturity studies EEG sleep studies and evoked potentials have been discussed elsewhere (17) Changes in the fundus of the eye have been reported to appear with advancing gestational age (24) The value of these changes for maturity assessment is not clear The same is true of reflex arc latency which recently has been shown to increase with advancing gestational age (10) Chemical analyses have shown changes related to gestational age for enzymes in the meconium (11) and serum enzymes (GPT) (22) Oxygen consumption studies have shown differences between pre term SGA and full term AGA infants (31) The level of immunoglobulin G increases with advancing age (2 33 35) SGA infants have decreased levels as compared with AGA infants of the same age (26 35) Total red cell volume increases with gestational age and the quotient total red cell volume/birth weight shows a slight decrease with advancing gestational age (5) For all these biochemical parameters the variation at a given gestational age is too great for them to be of any practical value in maturity assessment For further review of methods for maturity assessment see Casaer & Akiyama (7)

At present the following conclusion apply The *best methods for postnatal maturity assessment* are external characteristics or neurological tests see also Casaer & Akiyama (7) von Harnack & von Bernuth (20) Head circumference is also a valuable measurement The use of combinations of these methods increases the precision of estimating gestational age EEG sleep studies is an interesting method but it is time-consuming and needs to be further evaluated Further studies of fetal hemoglobin would also be of interest

SUMMARY

Five different methods for maturity assessment were applied to a material of 174 newborn infants of various gestational ages

The methods consisted of measuring or scoring the following parameters anthropometric measurements external characteristics neurological tests examination of epiphyseal centers and motor conduction velocity

Prediction models for estimating gestational age using single methods or combinations of methods are given

The best single methods were scoring of external characteristics and neurological tests the former being the most suitable for routine work during the first postnatal week

All methods except motor conduction velocity underestimate gestational age in small for gestational age infants

By combining methods the precision of estimating gestational age increases Useful combinations are any two or all of the following external characteristics neurological tests and head circumference

The precision of the prediction models was tested using a second material of 28 infants not included in the construction of the models The results of these tests indicate that it is possible to obtain a precision in the age estimation of $\pm 2^1/$ weeks for about 95 % of the infants (27 of 28 in this study) using a combination of methods

Factors limiting the precision of estimating gestational age are the time variation between LMP and conception the degree of reproducibility of the methods and the biological variation in maturity at a given gestational age

Various difficulties in evaluating the results of maturity studies are discussed It is pointed out that new methods must be compared with a simple and precise reference method based for example on birth weight applied to the same material Comparison between different methods applied to different materials must be made with caution and the commonly used 95% confidence limits are not suitable for such comparison

ACKNOWLEDGEMENT

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A THERMOGRAPHIC STUDY OF INFANTS EXPOSED TO COLD

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There is conclusive evidence that brown adipose tissue is the main site of heat production in response to cold exposure in newborn rabbits (5)

Human infants possess about 15 g of brown adipose tissue part of which is situated in the neck and between the scapulae (2) It has been demonstrated indirectly that brown adipose tissue in newborn babies is also influenced by cold Heim et al for instance found at post mortem examination a depletion of the lipid in brown fat cells of infants exposed to low ambient temperature during life (10)

Brown fat is richly vascularized and during cold exposure as well as during nor epinephrine infusion the blood flow through the tissue is greatly increased (9) thus dispersing the heat Heat, released by cold induced metabolism of the cervical and interscapular brown adipose tissue is transferred via direct venous connections to the vertebral sinus (14) In guinea pigs, a thermosensitive area in the cervical spinal cord has been found and outlined The warmed blood drained from the brown adipose tissue might protect vital nerve centres during cold exposure In these animals shivering is suppressed as long as the temperature of the cervical canal is maintained above a certain level (4)

Heat generated in brown fat cells might also be conducted to the body surface being thus accessible to measurement Silverman and more recently Grausz, associated the relatively high skin temperature of the nape of the neck of cold exposed infants to heat production in brown fat (8 13)

With the aid of thermography we examined the dorsal surface of neonates The purpose was to demonstrate if a cold induced increase in heat radiation appeared over areas where brown fat should be subcutaneously situated

Cytological examination of subcutaneous tissue was carried out in order to find out if there were any brown fat cells in the warm area in the nape of infants exposed to low ambient temperature

MATERIAL

The material consisted of 43 healthy infants having gestational ages of 38 to 44 weeks who were checked before the tenth day of life All infants examined were born after uncomplicated pregnancies and deliveries (vertex presentation) at the Sabbatsberg Hospital Stockholm Permission for each study was obtained from the parents who were informed about the details

Group A The first thermographic study performed included 19 infants with a birth weight of 2 750 to 3 820 g The babies were brought from the postnatal wards with an environmental temperature of about 24 C They were undressed and placed on their abdomen on a table in front of the thermo-

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This study was supported by grants from Karolinska Institutets Reservationsanslag and Sallskapet Barnavård

Part of this work (group A) was reported at the yearly conference of the Swedish Medical Society November 1968 (12)

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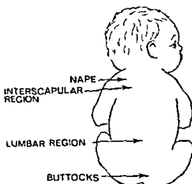


Fig 1 The points of temperature recordings on the back surface of the infants

graphic equipment for 30 min The ambient temperature was kept at about 21 to 22 C during the th rmographic recordings

Observations made during this study initiated further investigations under somewhat more controll d conditions

Group B Ten infants with a birth weight of 3 ?40 to 4 0?0 g were undressed and placed in an incubator (Isolette) at 3?-33 C for about 30 min As re tal temperatur rose during the stay in the incubator (B and C Fig 2) the environment there might b defined as a heat gaining one though th initial skin temperature of the infants was high r than th ambient temperature (1) Thus the infants metabolism should be at a basal level Thereafter they were kept on their abdomen on a table in front of th thermography camera for 30 min The ambient temperature was kept at 2? 23 C because this degree of cold exposure is found to be at the lower border of th control range Thus th infants should still be able to compensate for the heat loss (3) The cooling caused changes in skin t mperature without any apparent strain and the infants for the mo t part quietly sucked a teat

Group C Seven infants of low birth weight (1 860 to ?680 g) were treated in the sam way as infants of group B Birth weights and lengths were clo e to ? SD according to the diagram of Engstrom & Sterky (6) The neurological reflex pattern corresponded to that of fullterm infants (11)

Group D Seven infants (birth weight 2 980 to 4 3 0 g) were immersed in a water bath of 38 C and quickly dried with a towel before they were put in front of Thermovision As the evaporation increased the heat loss and cooled the body surface the difference in temperature change of various areas were potentiated. Photographs were taken after 5 15 and 30 min exposure to an ambient temperature of 23 C

METHODS

Thermography

An AGA Thermovision® unit System 660 was used to record the infrared emission of the body surface of the infants The equipm nt is made up of two basic units An infrared camera and a modified oscilloscope that displays the th rmal picture on a screen Thermography works according to a scanning principle utilizing the body s spontaneous infrared emission in the spectral region of 2-2 5 μ D tailed information is available in a general pamphlet distributed by th manufacturer AGA AB Lidingo Sweden (15)

In th thermal picture present d on the screen a warm area is lighter than a colder area Generally we used the inverted pres ntation that is warm regions appear darker than cold ones A built in isotherm display produces a selected temperature range shown as an area of saturated white superimposed upon the picture This isotherm delineates all areas of identical temperature within the selected range Th thermographic instrum nt can be calibrat d to a standard reference of a constant temperature By adjusting the ensitivity of the equipment and by movement of the isotherm scale temperature variation of 0 2 C may be shown

A mirror in front of the bed reflected the infrared emis ion from the baby into the camera situated about 2 m from the subj ct The reference temperature of 37 C was placed clo e to the baby in order to make possib e a correlation to the temperature of th skin During cold exposure r peated th rmographic recordings were made With a Minolta camera fitted to the display unit of the Thermovision photographs were taken Cold exposure began about 60 min aft r a meal The infants were carefully kept in a symmetrical position during the study We did not pay regard to the neck as the skinfolds there cause a cross radiation resulting in too high a temperature However we registered the temperatur chang s of the area between the neck and upp r border of the scapulae which we refer to as th nape (Fig 1)

Temperature recording

Rectal temperature at a depth of 6 cm and skin temperature were measured with an electric thermomet r type Ellab H_8 (Copenhagen) The skin applicator was applied to the body surface and the temperature was noted when it became stable (that is within 10 sec)

Temperature registrations were made in groups A B and C In A measurings were made after 5 and after 30 min of cold exposure In B and C recordings were made immediately after undressing after 30 min stay in the incubator after 15 and after 30 min of cold exposure

At each time of recording skin temperatures were measured after the thermographic exposure in order not to interfere with th heat emission

The registered skin areas are shown in Fig 1 Skin t mperatures of closely situated parts of the nape and th interscapular region varied mu h more than those of lower back areas A mean value of six measurements in the nape region was used to represent this ar a (Fig 2) Consequently the dif

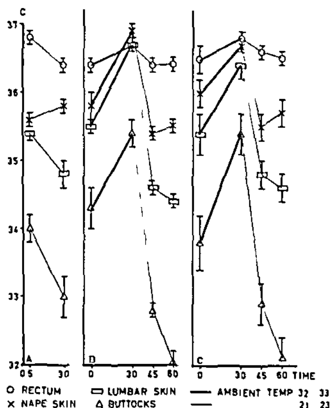


Fig 2 The change in temperature of infants during 30 min of exposure to an ambient temperature of 32–33 C (B and C) and during 30 min of exposure to an ambient temperature of 21–23 C (A B and C) Mean ±S E

ference in temperature between the upper and lower back surface seemed less pronounced than it would be if the warmest point of the nape alone was compared with the lumbar temperature

Cytology

With the aid of a Franzén puncture instrument and a fine needle for subcutaneous use aspiration biopsy was made from 10 infants (7) One sample of tissue was obtained from the part of the nape with the highest heat emission Another sample was taken from the buttocks The material was smeared out on a glass and stained with hematoxylin eosin for histological examination

RESULTS

In the majority of infants an area situated in the nape and interscapular region was the warmest one on the back surface after 30 min of exposure to cold

The temperatures of 2 infants of group A and one of group C were not included in the material The cold exposure had to be interrupted after 20 min as rectal temperature fell and a slight cyanosis became apparent There were no signs of an increased heat radiation of the nape in these babies (Table 1)

The results of temperature recordings of 17 infants of group A, all 10 infants of group B and 6 infants of group C are demonstrated in Fig 2

A tendency to increase in temperature of the nape skin was common for the three groups of infants As the skin of the neonates

Table 1 *The effect of cold exposure of neonates on heat emission of the nape*

Group	No	Mean birth weight (g)	*Type of cold exposure*	Duration of cooling (min) *20*	*30*	*40*	Unchanged or increased heat radiation from the nape at the end *of cooling*
A	19	3 340	After undressing exposure to 21–22 C	2	17		17
B	10	3 520	After undressing 1) 30 min exposure to 32–33 C 2) exposure to 22–23 C		10		10
C	7	2 270	After undressing 1) 30 min exposure to 32–33 C 2) exposure to 22–23 C	1	6		6
D	7	3 700	After undressing and quick immersion into 38 C water exposure to 22–23 C		5	2	7
Total	43			3	38	2	40

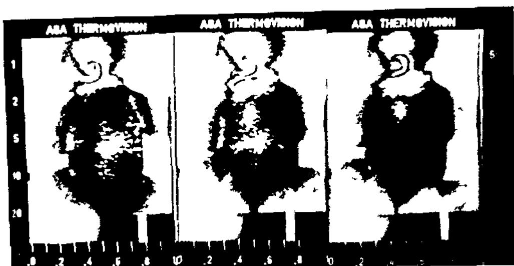


Fig 3 One infant after 5 15 and 30 min of exposure to co d Warm areas app ar darker than cold one (= inverted presentation) In using the isotherm func tion the areas on the back showing the high st heat emission have been covered by saturated white Thus it is seen that after 5 min of cooling (A) an area in the middle of the back is the warmest one After 15 min (B) the nape also shows th same degree of h at emission After 30 min of cold exposure (C) the nape and interscapular region alone form the warmest part of the back

in group B and C gained heat during their stay in the incubator their temperatures at the start of cooling were higher than those of A and the cooling caused an initial pronounced drop in temperature of the whole surface in cluding the nape During the last 15 min of cooling however a slow heat gain of the nape was recorded at the same time as lower skin areas continued to cool As a preliminary test skin temperature in 4 infants was recorded every fifth minute during cold exposure The lowest values of the nape temperature were seen within 10 min Thus when we in group B and C registered the temperature after 15



Fig 4 Heat radiation of one infant with a d layed reaction of the cold induced change of temperatures For technical description see text of Fig 3 After both 5 (A) and 25 min (B) cooling th lumbar area is warm r than the nape (C) After 40 min of cold exposure a small patch in th nape has gained heat

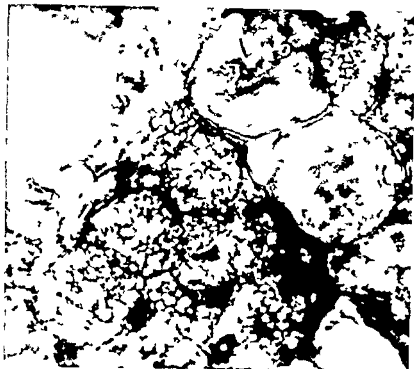


Fig 5 Multilocular fat cells obtained in the cold induced warm area on the back of an infant

min of cooling the nape should be in its heat gaining phase and the observed difference between 30 min and 15 min of cooling a minimum value

Infants of normal and low birth weight behaved in the same way though the level of temperature of the smaller babies was somewhat higher

Five infants of group D showed the pattern of reaction illustrated in Fig 3 After 5 min of cooling the lower back was warmer than surrounding regions 10 min later the warmth released by the nape and interscapular areas predominated and after 30 min the heat emission here seemed to have increased further

In 2 infants there was a delay in the cold induced reaction of the nape (Fig 4) which remained cooler than the lower back after 25 min As rectal temperature remained constant the cooling was extended another 15 min During this time the temperature of the nape increased and the final distribution of heat was more similar to that seen in the other infants of this group

In 6 out of 10 children samples of multilocular fat cells were found among unilocular ones in the subcutaneous tissue obtained from the warmest part of the nape (Fig 5) No collections of multilocular cells were found in the buttocks

In one third of all infants and in 4 of 7 babies of low birth weight a shivering like activity occurred at the end of the cooling period A few groups of muscles only were involved in the activity generally the thorax and thigh muscles

DISCUSSION

Thermographic recording of temperatures and the direct measurement using a skin applicator both fail in precision However with thermography an objective way of registration of the heat distribution is possible and by direct temperature measurements the estimated thermographic temperatures can be controlled The results in this study are not significant However the validity of the observed temperature changes is strengthened by the fact that the same cold induced reaction of the nape skin was found in most of the infants independently of treatment before cold exposure

Heat recorded by means of thermography emanates from the surface of the body but is

dependent on the metabolism of underlying tissues which are in circulatory connection with the skin A thick fat layer might impair the penetration of heat to the skin surface

In spite of a rather thick subcutaneous fat layer in the nape the skin temperature generally remains higher than that of lower areas This phenomenon could be due to a rich vascularization of the tissue Moreover as the nape and interscapular skin often gain heat during part of the cooling period the existence of a tissue with high metabolic activity in this region can not be excluded (8 13) The comparatively high heat radiation here might be an *indirect sign of non shivering thermogenesis going on* in deeper located tissue for instance in brown adipose tissue

In most infants a tendency to heat gain in the nape region was first noticed after 10 to 15 min of exposure to cold This pattern of reaction corresponds well in time with the start of the metabolic rise during exposure to the same ambient temperature noticed by Bruck (3)

The low heat radiation of the nape seen in 5 infants could perhaps be due to a peripheral vasoconstriction induced by hypoxia (3) It might also be explained by an insufficient perfusion of oxygen through brown adipose tissue thus impairing the oxidation of FFA and causing a reduction of the heat release

The samples obtained of multilocular cells from the warmest part of the nape of neonates support the theory that heat released there during cold exposure depends on an increased metabolic activity of brown adipose tissue Only one aspiration biopsy was made from each region of each child Cells of white adipose tissue mingle with cells of brown adipose tissue in the subcutaneous layer (2) Moreover brown fat cells rich in lipid are unilocular and very difficult to distinguish from white ones These conditions make it hard to conclude whether in those infants where no multilocular cells were found there really was a lack of brown fat cells

In accordance with Adamsons et al we observed shivering in some cold exposed infants (1) This muscle activity was more frequently seen in infants of low birth weight than in those of normal birth weight

SUMMARY

Forty three healthy fullterm infants 1 to 10 days old were studied with thermography during 30 min of exposure to an ambient temperature of 21 to 23 C

In most of the infants an area situated in the nape and interscapular region was the warmest one on the back surface after 30 min of exposure to cold In this region there was a tendency to an increase in temperature simultaneous to continued cooling of the lower back areas during exposure to low ambient temperature

In 5 infants there was a lack or delay of the heat gain in the nape and the interscapular region during cold exposure Three of these infants showed signs of hypoxia

In 6 of 10 infants collections of multilocular cells were found among the white adipose tissue in that part of the back which was warmest after cold exposure

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JUVENILE GOITROUS AUTOIMMUNE THYROIDITIS

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In recent years it has become evident that autoimmune thyroiditis (AIT) is by no means a rare disease in children and adolescents (23 33 34 38) It is not clear whether the apparently increasing prevalence of AIT[1] is due to better diagnostic tools and increasing interest in the disease or to a real increase in morbidity The first alternative seems more probable

The purpose of this paper is to describe the clinical and immunological findings in 22 goitrous children with AIT The radioiodide studies and investigation of intrathyroidal iodine and protein distribution have been presented elsewhere (26 31 32)

MATERIAL AND METHODS

Patients

Th series consists of two groups of consecutive patients with AIT and goitre remitted to the Children s Hospital University of Helsinki from January 1962 to February 1969 Group I (Table 1) comprises 13 euthyroid and Group II (Table 2) nine hypothyroid girls

Hereditary factors

All the parents were questioned about the occurrence of thyroid diseases in the family Eighteen of the mothers and nine of the fathers were examined clinically and PBI BEI thyroid antibodies and serum chol sterol were measured

Methods

The diagnosis of AIT was based on morphological and/or serological criteria Both histological and cytological techniques were used Specimens for histology were obtained by open biopsy and the amount of lymphocytic infiltration was consid red indicative of the disease Cytological specimens were obtained by the fine needle technique and were evaluated as described by Persson (40)

Two types of antibodies were determined anti thyroglobulin with th TRC and antimicrosomal with the CF test (Department of Serology and Bacteriology University of Helsinki) A TRC titre of 1/25 000 or higher or a CF titre of 1/32 or higher was regarded as diagnostic when a positive biopsy was not available (except in patient No 14 who had a TRC titre of 1/2 500 but a firm goitre) Th diagnosis of euthyroidism and hypothyroidism was based on the results of clinical examination and of several thyroid function tests (29) except for the radioiodide t sts and the PBI which may give erroneous results in AIT For the diagnosis of hypothyroidism the generally accepted symptoms and signs were required Some symptoms and signs on which the diagnosis of thyroid function was based are presented in Table 3

The thyroid uptake of radioiodide (^{131}I) was measured with a scintillation detector in most instances 2½ 6 24 48 and 72 hr after an oral dose of 5–40 μCi of the isotope The plasma samples and the urinary excretion of radioiodide were measured in a well typ scintillation detector for 24 48 and 72 hr after administration of the isotope PBRI was measured with Ioresin® tubes (Abbot Lab Inc USA) and NBERI after extraction with butanol acidified with H_2SO_4 according to the method of Escobar del Rey et al (1?) The methods for the perchlorate discharge test and for the thyrotropin stimulation test are presented elsewhere (31) PBI was determined either by the method of Foss et al (16) or with a Technicon Auto-Analyzer BEI by th method of Fisch r et al (14) serum cholesterol by the method of Ness et al (37) and HOP as described by Kivirikko

[1] The following abbreviations will be used AIT = autoimmune thyroiditis BA = bone age BEI = butanol-extractable iodine CA = chronological age CF = complement fixation HA = height age HOP = hydroxyproline NBERI = non butanol-extractable radioactive iodine PBI = protein bound iodine PBRI = protein bound radioactive iodine TRC = tanned red cell agglutination TSH = thyroid stimulating hormone

This study was made possible by a grant from the Paulo Foundation

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Th series consists of two groups of consecutive patients with AIT and goitre remitted to the Children s Hospital University of Helsinki from January 1962 to February 1969 Group I (Table 1) comprises 13 euthyroid and Group II (Table 2) nine hypothyroid girls

Hereditary factors

All the parents were questioned about the o currence of thyroid diseases in the family Eighteen of the mothers and nine of the fathers were examined clinically and PBI BEI thyroid antibodies and serum chol sterol were measured

Methods

The diagnosis of AIT was based on morphological and or serological criteria. Both histological and cytological techniques were used Specimens for histology were obtained by open biopsy and the amount of lymphocytic infiltration was consid red indicative of the disease Cytological specimens were obtained by the fine needle te hnique and were evaluated as described by Persson (40)

Two types of antibodies were determined anti thyroglobulin with th TRC and antimicrosomal with the CF test (Department of Serology and Bacteriology University of Helsinki) A TRC titre of 1/25 000 or higher or a CF titre of 1 32 or higher was regarded as diagnostic when a positive biopsy was not available (except in patient No 14 who had a TRC titre of 1/2 500 but a firm goitre) Th diagnosis of euthyroidism and hypothyroidism was based on the results of clinical examination and of several thyroid function tests (29) except for the radioiodide t sts and the PBI which may give erroneous results in AIT For the diagnosis of hypothyroidism the generally accepted symptoms and signs were required Some symptoms and signs on which the diagnosis of thyroid function was based are presented in Table 3

The thyroid uptake of radioiodide (^{131}I) was measured with a scintillation det ctor in most instances 2, 6 24 48 and 72 hr after an oral dose of 5–40 μCi of the isotope The plasma samples and the urinary excretion of radioiodide were measured in a well type scintillation detector for 24 48 and 72 hr after administration of the isotop PBRI was measured with Ioresin® tubes (Abbot Lab Inc USA) and NBERI after extraction with butanol acidified with H_2SO_4 according to the method of Escobar del Rey et al (12) Th methods for the perchlorate discharge test and for the thyrotropin stimulation test are presented elsewhere (31) PBI was determined either by the method of Foss et al (16) or with a Technicon Auto-Analyzer BEI by th method of Fischer et al (14) serum cholesterol by the method of Ness et al (37) and HOP as described by Kivirikko

[1] The following abbreviations will be used AIT = autoimmune thyroiditis BA = bone age BEI = butanol-extractable iodine CA = chronological age CF = complement fixation HA = height a e HOP = hydroxyproline NBERI = non butanol-extractable radioactive iodine PBI = protein bound iodine PBRI = protein bound radioactive iodine TRC = tanned red cell agglutination TSH = thyroid stimulating hormone

This study was made possible by a grant from the Paulo Foundation

Table 1 *Clinical data on 13 euthyroid girls with autoimmune thyroiditis (Group I)*

Patient No	Age at time of diagnosis	Heredity	Estimated duration of disease (yrs)	Thyroid gland			
				Estimated size (g)	Nodules	Consistency	Scintigram
1	15 1	Non-contributory Both parents examined normal	2	35		Firm	Diffuse enlargement activity even
2	13 8	Maternal grandmother has thyrotoxicosis a paternal aunt hypothyroidism Mother examined normal	0 1	Normal–slightly enlarged		Firm	
3	13 3	Non-contributory Both parents examined normal	1	40		Soft	
4	11 8	Father has AIT and hypothyroidism Mother s TRC 1/250 Maternal grandmother operated on because of goitre two maternal aunts have goitre	1	25		Firm	Left lobe larger activity even
5	11 8	Non contributory Both parents examined normal	0 3	35	Multinodular	Firm	Left lobe more active
6	11 8	A maternal aunt had goitre at puberty	4	70	Multinodular	Normal	
7	11 2	Non contributory	0 2	45		Firm	
8	9 8	Mother TRC 1/25 CFT 1/8	0 1	30		Firm	Normal
9	9 7	A maternal aunt has goitre Both parents examined normal	1 3	30		Right lobe firm left lobe normal	Normal
10	9 7	Mother has AIT a paternal aunt operated on because of thyrotoxicosis	0 1	35		Right lobe larger firm	Active nodule in right lobe
11	9 3	Mother and 3 of her sisters have AIT	0 1	30		Firm	Normal
12	8 9	Father PBI 5 1 BEI 3 4 TRC 1/2500 mother normal	0 3	30	One nodule in right lobe	Firm	
13	7 8	Mother has AIT a maternal aunt operated on because of goitre	2 0	25		Firm right lobe larger	

et al (27) The quantitative determination of serum protein was made by the biuret method and for cellulose acetate electrophoresis the Beckman Microzone System® was used The immunoglobulins were determined by a method described by Immonen (24)

HA was given with reference to the standards of the Finnish Centre for Study of Child Growth and Development and the BA to those of Greulich & Pyle (17)

PBI (µg/100 ml) (4 0–8 0)	BEI (µg/100 ml) (3 5–7 5)	Cholesterol (mg/100 ml)	HOP[a] (mg/24 hr)	Highest titres of thyroid antibodies TRC[b]	CF	Biopsy finding	Remarks
5 9	4 2	295	70	1/25 000	1/512		Atopic eczema asthma from the age of 5 yrs
22 0–6 2	4 5	92–152	65	1/2 500 000	—		Subacute hyperthyroid onset Later rheumatoid arthritis, malabsorption syndrome and autoimmune nephropathy
9 9	2.2	230	117	1/5	1/4	+	A nodule was found in the left lobe at operation
4 9	2.7	282	55	1/25	1/32	+	
5 8	4 1	192	124	1/25	1/64	+	
6 3–4 6	4 4	190		1/2 500 000	1/128		Thrombocytopenic purpura
6 7		270		1/25	1/32		Atopic eczema since infancy hay fever
8 0–9 8	5 9	17		1/250 000	1/512	+	Hyperthyroid onset Collagenosis 1 yr later Emotionally disturbed
8 3	7 3	191		1/2 500 000	—	+	
6 7–6 4	4 0–2 9	181	75	1/250	—	+	
8 2		135		1/25 000	1/128	+	
8 1	5 7	333	63	—	1/64	+	Atopic eczema A nodule was found in both the right and the left lobe at operation
8 1	5 4	223	43	1/250	—	+	Diabetes mellitus from the age of 3 yrs

[a] Hydroxyproline excretion in urine For normal values see ref No 48
[b] Antithyroglobulin antibodies tanned red cell agglutination
Antimicrosomal antibodies complement fixation
Normal values in brackets

Table 2 *Clinical data on 9 hypothyroid goitrous girls with autoimmune thyroiditis (Group II)*

Patient No	Age at time of diagnosis	Heredity	Estimated duration of disease (yrs)	Thyroid			
				Estimated size (g)	Nodules	Consistency	Scintigram
14	13 1	Non-contributory	2 0	25		Isthmus firm other wise normal	Activity even
15	12	Mother had goitre at puberty	3 5	30		Firm	
16	10 8	Mother s TRC 1/250 Paternal grand mother operated on because of toxic goitre A paternal aunt treated for thyrotoxicosis	0 3	25		Firm	Right lobe larger activity even
17	10 8	A paternal aunt operated on because of Graves disease Mother had goitre at puberty Both parents examined normal	1	80		Normal	
18	10 5	Father operated on because of Graves disease Both parents examined and now normal	1	30		Firm	Left lobe larger activity even
19	10 4	Non contributory Mother examined normal	4	35		Firm	Left lobe normal scattered activity in right one
20	10 2	Non-contributory Mother examined normal	3	30		Firm	
21	8 8	Mother has AIT Father PBI 10 4 BEI 4 6 no thyroid antibodies A paternal aunt has goitre	0 3	35	Isthmus most en larged	Firm	Active nodule in isthmus
22	5 8	Maternal grand mother has hypo-thyroidism Mother examined normal	0 5	70	2 nodules	Normal	

RESULTS

Heredity

Group I Six of the fathers and 11 of the mothers were examined Three of the mothers (Nos 10, 11 and 13) and one of the fathers (No 4) had verified AIT and two of the mothers (Nos 4 and 8) and one of the fathers (No 12) had evidence of thyroid autoimmunity Thus there was evidence of thyroid auto immunity in six of the 13 families

Group II Three of the fathers and seven of the mothers were examined One of the fathers (No 18) had been operated on because of Graves disease but now he was euthyroid and results of the laboratory tests were normal In one of the mothers (No 21) AIT and in an

PBI (/ g/100 ml) (4 0–8 0)	BEI (/ g/100 ml) (3 5–7 5)[a]	Cholesterol (mg/100 ml)	HOP[b] (mg/24 hr)	Highest titres of thyroid antibodies TRC[c]	CF[d]	Biopsy finding	Remarks
2 1	1 0	222	45	1/2 500	—		
5 3– 2 2		388– 700		1/5	1/128		
9 1– 8 9	4 0	152– 254	70– 61	1/250 000	1/3[illegible]		Hyperthyroid onset
8 [illegible] 3 1		310– 508		1/25	1/128	+	
3 2		284	23	1/2 500	1/64		Diabetes mellitus from the age of 8 yrs
6 9– 2 2		288– 318		1/5	1/16	+	
[illegible]0– 1 [illegible]		332– 470	5	—	1/64		Diabetes mellitus from the age of 1 8 yrs and malabsorption syndrome from 2.5 yrs
5 3 4 7	1 9– 1 0	205	30	1/[illegible]5	1/256	+	Atopic eczema
4 7		250	23	—	1/64		Atopic eczema and hay fever

[a] Normal values
[b] Hydroxyproline excretion in urine For normal values see ref No 48
[c] Antithyroglobulin antibodies tanned red cell agglutination
[d] Antimicrosomal antibodies complement fixation

other one (No 16) a TRC titre of 1/250 were detected Thus evidence of autoimmune thyroid disease was found in three of the nine families

More information are given in Tables 1 and 2

Age and sex

All the patients both euthyroid and hypothyroid were girls Most of them were in pre puberty or puberty The age range was from 7 8 to 15 1 (median 11 2) years in Group I and from 5 8 to 13 1 (median 10 5) years in

Table 3 *Symptoms and signs of 13 euthyroid (Nos 1–13) and 9 hypothyroid (Nos 14–22) goitrous girls with autoimmune thyroiditis*

	1	2	3	4	5	6	7	8	9	10	11	12	13
Age at the time of diagnosis	15 1	13 8	13 3	11 8	11 8	11 8	11 2	9 8	9 7	9 7	9 3	8 9	7
Cold intolerance	0	0	0	0	0	0	0	0	+	0	0	0	0
Constipation	0	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue and dullness	0	0	0	0	+	+	+	+	+	0	0	0	0
Cool pale skin	0	0	0	0	0	0	0	+	0	0	+	0	0
Dry skin	0	0	0	0	0	0	+	0	0	0	0	0	0
Puffy features	0	0	0	0	0	0	0	0	0	0	0	0	0
Hoarseness	0	0	0	0	0	0	0	0	0	0	0	0	0
Slow movements	0	0	0	0	0	0	0	+	0	0	0	0	0
Delayed return of reflexes (Examined clinically)	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth retardation	0	0	±	+	0	0	0	0	0	0	0	0	0
Bone age		17	11½	8¾	12	10½			10	7¾		10	8½

Group II Thus the age distribution was very similar in the two groups

Onset and symptoms

The onset of the disease was evaluated from the history and the growth curve

Group I The mean duration was 0 9 years (0 1–4 years) Most of the children had very few if any definite symptoms of thyroid disease except for the goitre The presence of a goitre was first observed on health examination or on consultation for other reasons in seven patients

Three girls were irritable or nervous and two had increased sweating symptoms which may equally well be associated with the approach of puberty Other reasons for admission were idiopathic thrombocytopenia with haemorrhages (No 6) and failure to grow (No 4) The parents of the last mentioned patient were of short stature and the patient herself had been short since the age of 2½ years and had not gained any height in the last year She may have had borderline hypothyroidism but was considered to be clinically euthyroid In patient 8 initially Graves disease was suspected but the disease turned out to be AIT Patient 2 had hyperthyroidism and subacute thyroiditis Her course is interesting from the aetiological point of view She may be an example of general breakdown of immunological tolerance The patient had the typical subacute thyroiditis of de Quervain with thyrotoxic onset but histological confirmation is lacking Serological tests for several virus antibodies and the thyroid antibody titres were negative On administration of prednisone the symptoms subsided and the size of the thyroid normalized A year after the first attack she suffered a relapse with milder symptoms Half a year later she had another relapse the TRC titre now being 1/250 000 Her ESR remained elevated at the level of 40 mm/hr and some months later she presented with rheumatoid arthritis with positive Wailer Rose and Latex tests and antinucleαr antibodies She had autoimmune nephropathy and subtotal villous atrophy of the small intestine too

Group II In this group the onset of the disease was more insidious than in Group I The mean duration was 1 7 years (0 3–4 years) Patient 16 had a subacute onset with thyrotoxic symptoms Her symptoms subsided without treatment in the course of half a year after which she began to complain of symptoms attributable to hypothyroidism fatigue cold intolerance and loss of appetite Five patients were referred because of symptoms of hypothyroidism In three girls the main reason for pediatric consultation was swelling of the neck One of them (No 21) had only slight signs of hypothyroidism while another (No 17) was slightly hypothyroid when first seen

14	15	16	17	18	19	20	21	22
13	1?	10 8	10 8	10 5	10 4	10 2	8 8	5 8
0	+	+	+	+	+	+	0	0
+	–	0	+	+	+	+	0	+
+	+	+	+	+	+	+	+	+
+	–	+	+	+	+	+	+	+
+	+	+	0	+	+	+	+	+
+	–	0	+	+	+	+	+	+
0	0	0	+	+	0	+	0	0
–	0	+	+	+	0	–	0	–
+		+	+	+	+	+	+	0
+	–	0	0	+	+	+	0	0
11	10	10½	9	8½	11	5	8½	5½

The latter was discharged without treatment and two months later was admitted for further investigation and during that interval full blown myxoedema had developed The third patient (No 15) was observed for two and a half months before the institution of the treatment during that time her mild hypothyroidism developed into obvious myxoedema

Clinical signs

Goitre The most conspicuous sign in Group I was the goitre The thyroid was slightly or moderately enlarged in most instances only

Table 4 *Radio iodide studies on 13 euthyroid (Group I) and 9 hypothyroid (Group II) girls with autoimmune thyroiditis*

Patient No	PBI (µg/100 ml) (4–8)	BEI (µg/100 ml) (3 5–7 5)	PBRI 72 hr (% dose/l) (<0 3)	NBERI[b] (of PBRI) (<20)	U_t (dose) (16–41)	T½ (>35 d)	TSH[c] test	Perchlorate test (decrease)
Group I								
1	5 9	4.2	0 09	73	47			
2	22 0–		0 63		2–			
	6 2	4 5	0 64	10	36	5 1		No
3	9 9	2.2	1 06					
4	4 9	2 7	0 24		28		–	
5	5 8	4 1	0 26	34	34	Normal	±	
6	6 3–							
	4 6	4 4	0 59		33	2 5		
7	6 7		1 16	58	33	8 1		
8	8 0–							
	9 8	5 9	2 30		54			
9	8 3	7 3	0 44	51	54	7 0		
10	6 7–	4 0–	0 83–	46	60–	6 2–		
	6 1	2.9	1 02		23	3 5	–	41
11	8 2		0 24	45	31	Normal		
12	8 1	5 7	0 140					
13	8 1	5 4	0 67		27 2	10 5		
Group II								
14	2 1	1 0	0 338	81	7 1	3 0	–	
15	5 3–							
	2 2							
16	9 1		0 231	52	15	2 3	–	
	8 9	4 0						
17	8 7		0 21	29	43			
			0 94–	63	63	17–		
	3 1		0 90					
18	3 2				25	2 2	–	33
19	6 9–		0 26	31	23	4.5		
	2 2	0 5	0 01					
20	2 0–				9	1.5	–	26
	1 6							
21	5 3–	1 9	0 14	22	8	1 0		
	4 7	1 0						
22	4 7		1 75	22	41	3 5	–	83
			2 83		39	3 0		

Protein bound radioactive iodine
[b] Non-butanol-extractable radioactive iodine
Biological half life of the thyroidal radio-iodine
[c] – No response ± PBI increased 1 µg/100 ml and the 24 h uptake from 34 to 46
Normal values in brackets

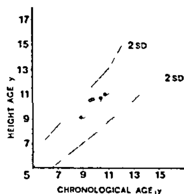


Fig 1 The height age at the time of diagnosis of 13 euthyroid (○) and 9 hypothyroid (●) juvenile patients with autoimmune thyroiditis

one gland was considered large with an estimated weight of 70 g In eight cases the gland was felt on palpation to be firm throughout in three patients only one lobe was firm and one gland was soft

Two glands were multinodular In one a single nodule was palpable but at operation another nodule was found In one goitre which on palpation appeared diffuse a single nodule was found at operation

Group II Two patients had large goitres two slightly enlarged and five medium sized glands In one patient it was mainly the isthmus that was enlarged and in another the left lobe was larger and softer than the right One gland contained two nodules Six goitres were firm and in one patient the thyroid was partly normal partly firm

Five glands out of 22 were nodular

Growth and development

HA and BA are shown in Figs 1 and 2

Group I In patient 3 skeletal maturation was delayed by nearly two years and growth was also slightly retarded Patient 4 had one year s stagnation of growth and her BA was three years delayed It should be noted that in these two patients the BEIs were 2 2 and 2 7 μg/100 ml They probably had borderline hypothyroidism although they were considered euthyroid on clinical examination and other laboratory tests were normal In patient 10 the BA was delayed by two years but her growth was normal and she had no symptoms or signs of hypothyroidism Patients 2 and 12 had BA more than one year advanced Two patients (Nos 1 and 2) had had the menarche and four patients (Nos 3 5, 6 and 7) had early signs of puberty

Group II Only one patient was clearly stunted although most individual growth curves showed some stagnation The BA was equal to the CA in three patients One of them (No 16) had had a subacute onset with hyperthyroidism and the other two (Nos 21 and 22) had a short history of disease Surprisingly patient 19 had normal HA and BA despite a four years history The explanation for this lay in the fact that the development of the patient was well in advance of the average before the onset of AIT None of these girls had had the menarche Patients 14 and 15 had early signs of puberty

Laboratory data

PBI and BEI Group I PBI was slightly elevated or high normal with a mean value of 8 3 μg/100 ml The mean BEI was 4 7 μg/100 ml in two patients it was below 3 0 μg/100 ml A significant difference (>1 5 μg/100 ml) between the PBI and BEI levels was observed in nine out of 11 cases Group II The mean PBI was 3 7 μg/100 ml the level first found was within the normal range in six patients The BEI was only determined in

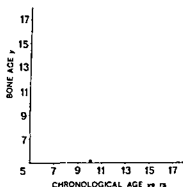


Fig 2 The relation between bone age and chronological age of 9 euthyroid (○) and 9 hypothyroid (●) juvenile patients with autoimmune thyroiditis

three cases In one it was normal at the first determination

Radio iodide test The results are summarized in Table 4 Group I The uptake values were generally in the high normal range and in five patients above the upper normal limit Only in one patient was the uptake subnormal when first measured but later there was an increase (No 2) The biological halflife of the radio-iodine in the thyroid gland was significantly decreased in seven patients and normal (>35 days) in two The PBRI was clearly elevated (≥0 3° /1) at 72 hr in eight cases and normal in five The NBERI was at a significant level (≥25 of PBRI) in six out of seven patients Group II The uptake was elevated in one normal in five and subnormal in three patients The biological half life of radio-iodine in the thyroid gland was very short in all patients but the PBRI was elevated in only four out of nine patients The NBERI was determined in seven cases and was elevated in five of them TSH stimulation provoked no response in four and in patient

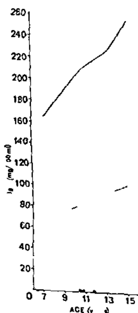


Fig 3 Serum levels of IgA in patients with autoimmune thyroiditis (O – euthyroid ● – hypothyroid) The interrupted line indicates the mean and the solid line the 97 5% confidence limit of normal values

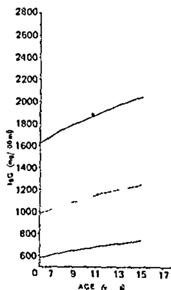


Fig 4 S rum levels of IgG in patients with autoimmune thyroiditis (O – euthyroid ● – hypothyroid) The interrupted line indicates the mean and the solid lines the 97.5 and 2.5% confidence limits of normal values

5 PBI increased 1 µg/100 ml and the 24 hr uptake from 34 to 46% after administration of 10 IU TSH intramuscularly

Excretion of hydroxyproline in the urine In recent years the HOP excretion in the urine has proved to be one of the best tests reflecting the peripheral action of thyroid hormones The normal values are age dependent (48) The reliability of this test is high in cases of hyperthyroidism in adult patients and in cases of hypothyroidism in children (48) In Group I it was normal in eight patients studied In Group II it was determined in six patients and was subnormal in five In one patient (No 16) it was within the normal range

Cholesterol Serum cholesterol was over 300 mg/100 ml in one out of 13 patients in Group I and four out of nine in Group II

Erythrocyte sedimentation rate In Group I seven had a normal (below 10 mm/hr) and six an elevated ESR In Group II the respective figures were two and seven

Thyroid antibodies The findings are summarized in Tables 1 and 2 Of the 13 patients

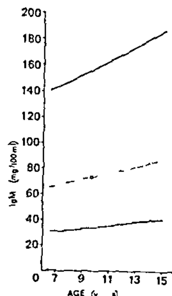


Fig 5 Serum levels of IgM in patients with auto immune thyroiditis (○ = euthyroid ● = hypothyroid) The interrupted line indicates the mean and the solid lines the 97 5 and 2 5 confidence limits of normal values

of Group I six had significant serum levels of TRC antibodies and eight of CF antibodies detected some time during the course of the disease Three patients had only low titres during the time of follow up Of the nine patients of Group II one had a significant serum level of TRC antibodies and seven of CF antibodies One patient had a TRC titre of 1 2500 and another had a CF titre of 1 16 which can both be regarded as almost significant in children Of these 22 patients with confirmed AIT a significant TRC titre was found in 15 (68 %) By the use of these two technique it was possible to establish the diagnosis in 17 out of 22 patients (77 %) There was a considerable variation in titre among determinations made at different times During the first half year of follow up only eight patients had significantly positive titres to thyroid antibodies At times a positive titre was detected after the initiation of thyroxine therapy or after a thyroid biopsy The number of antibody positive cases is higher if the test is repeated frequently No correlation was found between the antibody titre and the size of the thyroid or duration of the disease before diagnosis Thyroxine treatment had no influence on the level of circulating thyroid antibodies

Serum proteins and immunoglobulins The serum total protein was elevated above the normal mean value +2 S D for age in one patient of Group I and in five patients of Group II The mean was 7 6 g/100 ml in the former and 8 2 g/100 ml in the latter group Unfortunately adequate electrophoretic data are only available for half the patients The serum albumin was normal in nine and elevated above the mean +2 S D normal for age in two out of 11 patients in both groups Decreased levels of serum albumin as reported by others (36 44) could not be found The serum gammaglobulin was elevated in eight and normal in three patients

Serum immunoglobulins A G and M were quantitated in 17 (Figs 3 4 and 5) and in addition a semiquantitative determination was made in two patients at the time of diagnosis Three patients had deficiency and one patient a very low serum concentration (6 25 mg/100 ml) of IgA In six patients the IgG concentration was above the 97 5 % confidence limit of normal values and in two additional patients the semiquantitative determination gave abnormally high values Only one patient had an IgM concentration above the 97 5 % and one below the 2 5 % confidence limits of normal values

DISCUSSION

Recently attention has often been drawn to the increased frequency of thyroiditis This has been attributed to better diagnostic tools an increased awareness of the disorder or a real increase in the frequency of this disease Hereditary factors seem to play a role in the aetiology of AIT (6 10 15 19 20 47) but the pattern of transmission is not clearly understood It has been shown that predisposition to thyroid antibody formation is transmitted as a dominant trait with equal incidence in both sexes (20 21) but the preponderance of females in AIT is very great as only about

7 % of the cases in children are male (23) Involvement of the sex chromosome has been suggested (49) and some observations point to the X chromosome (11) All the patients in the present series were female

The subacute thyroiditis of de Quervain which is possibly caused by a virus infection (13) is presumably in some cases the first manifestation of an autoimmune disease Both the experimental data of van Loghem (35) and Eylan s (13) findings of the changes in the histological picture of subacute thyroiditis support this view In the present series patient 2 had the typical subacute thyroiditis before the occurrence of circulating thyroid anti bodies

Some clinical features of the cases of AIT merit discussion Three patients were hyper thyroid at the onset of the disease They had slight eye signs too resembling Graves dis ease The hyperthyroid symptoms subsided in a month or two and patient 16 developed clin i al hypothyroidism six months later This course does not appear to be common (9 38 42) although it occurred in about half the adult patients in the series of Scazziga et al (43) Such cases may be mistaken for Graves disease if the antibody titre is low and histological or cytological findings fail to give a clear picture of lymphocytic thyroiditis The triiodothyronine suppression test may be very useful in the differential diagnosis

On comparing the euthyroid (Group I) with the hypothyroid patients (Group II) it is evident that the two conditions have many features in common In both groups many patients had a strong family history of thyroid disease the age distribution was identical and the patients were females There was no clear difference in the size or consistency of the goitres The mean known duration of the disease prior to medical attention was longer (1 7 years) in the hypothyroid group than in the euthyroid group (0 9 years) although the euthyroid pa tients had fewer symptoms and complaints

A large difference between PBI and BEI in plasma is characteristic of Hashimoto s dis ease (4 18 19 36 42) but it is seen in other conditions as well in subacute thyroiditis (25) in Graves disease (45) in congenital goitrous hypothyroidism (7) in endemic goitre (30) in euthyroid goitrous patients (5) in thyroid car cinoma (46) after radio-iodide therapy (41) and in Down s syndrome (8) In the euthyroid group the difference was seen in 9 out of 11 patients and in the hypothyroid group it was only determined in three patients and was present in two of them

The thyroid uptake values were generally higher in the euthyroid than in the hypothyroid group In both groups one patient had a scintigram in which one lobe was more ac tive than the other and another a scintigram with "an active nodule" The triiodothyrodine suppression test was not done since there was no reason to believe that these were real autonomous lobes or nodules It was more likely that these lobes and nodules represented rests of the normal functioning thyroid tissue in glands in which the other parts had been damaged by thyroiditis

The thyroid antibody levels the serum protein findings and immunoglobulins were sim ilar in the two groups The serum gamma globulin was elevated in eight out of 11 pa tients The serum total protein was elevated more often (5/9) in hypothyroid patients than in euthyroid patients (1/13)

An abnormally high IgG serum concentra tion was found in eight patients and in 16 pa tients it was above the mean for the age It can be considered an indication of an active immunological process Isolated IgA defi ciency was found in three patients Previously it has been described in connection with con nective tissue diseases (2) and other autoim mune diseases (22) and only in three patients with thyroid disorders (1 3)

It is interesting that five of our 22 patients with AIT also had allergic manifestations mainly atopic eczema possibly reflecting an al teration in the immunological system The oc currence of other organ antibodies gastritis malabsorption syndrome and other associated

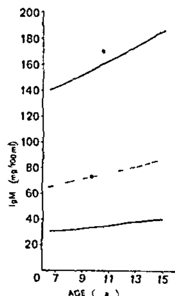


Fig 5 Serum levels of IgM *in patients with auto immune thyroiditis* (O – euthyroid ● – hypothyroid) The interrupted line indicates the mean and the solid lines the 97 5 and 2 5 confidence limits of normal values

of Group I six had significant serum levels of TRC antibodies and eight of CF antibodies detected some time during the course of the disease Three patients had only low titres during the time of follow up Of the nine patients of Group II one had a significant serum level of TRC antibodies and seven of CF antibodies One patient had a TRC titre of 1 2500 and another had a CF titre of 1 16 which can both be regarded as almost significant in children Of these 22 patients with confirmed AIT a significant TRC titre was found in 15 (68%) By the use of these two technique it was possible to establish the diagnosis in 17 out of 22 patients (77%) There was a considerable variation in titre among determinations made at different times During the first half year of follow up only eight patients had significantly positive titres to thyroid antibodies At times a positive titre was detected after the initiation of thyroxine therapy or after a thyroid biopsy The number of antibody positive cases is higher if the test is repeated frequently No correlation was found between the antibody titre and the size of the thyroid or duration of the disease before diagnosis Thyroxine treatment had no influence on the level of circulating thyroid antibodies

Serum proteins and immunoglobulins The serum total protein was elevated above the normal mean value +2 S D for age in one patient of Group I and in five patients of Group II The mean was 7 6 g/100 ml in the former and 8 2 g/100 ml in the latter group Unfortunately adequate electrophoretic data are only available for half the patients The serum albumin was normal in nine and elevated above the mean +2 S D normal for age in two out of 11 patients in both groups Decreased levels of serum albumin as reported by others (36, 44) could not be found The serum gammaglobulin was elevated in eight and normal in three patients

Serum immunoglobulins A G and M were quantitated in 17 (Figs 3 4 and 5) and in addition a semiquantitative determination was made in two patients at the time of diagnosis Three patients had deficiency and one patient a very low serum concentration (6 25 mg/100 ml) of IgA In six patients the IgG concentration was above the 97 5% confidence limit of normal values and in two additional patients the semiquantitative determination gave abnormally high values Only one patient had an IgM concentration above the 97 5% and one below the 2 5% confidence limits of normal values

DISCUSSION

Recently attention has often been drawn to the increased frequency of thyroiditis This has been attributed to better diagnostic tools an increased awareness of the disorder or a real increase in the frequency of this disease Hereditary factors seem to play a role in the aetiology of AIT (6 10 15 19 20 47) but the pattern of transmission is not clearly understood It has been shown that predisposition to thyroid antibody formation is transmitted as a dominant trait with equal incidence in both sexes (20 21) but the preponderance of females in AIT is very great as only about

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diseases in our patients will be discussed in another paper (28)

Of the present series of 22 patients with AIT, nine were hypothyroid Follow up studies indicate that a considerable proportion of the patients will develop hypothyroidism or re appearance of the goitre when treatment is discontinued (38, 43) Therefore treatment with *l* thyroxine was started in all patients as soon as AIT was diagnosed

SUMMARY

Clinical laboratory and immunological data on 22 juvenile patients with autoimmune thyr oiditis are presented All patients were female and nine were hypothyroid Three had a hyper thyroid onset of the disease The diagnosis was based on typical morphology and on signifi cantly elevated titres of antithyroglobulin or antimicrosomal antibodies There was a history of thyroid disease in nine of the families Every patient had a goitre of moderate or small size It was mostly firm and in five it was nodular The euthyroid patients had few if any symptoms except the goitre Of the hypothyroid patients only one was clearly stunted but most growth curves showed some stagnation and bone age was retarded in six PBI and BEI were significantly different in nine out of 11 patients protein bound radio active iodine was elevated in 12 out of 22 non butanol extractable radioactive iodine in creased in 13 out of 16 and T½ biol decreased in 16 out of 18 Of 19 patients eight had ab normally high IgG levels and three IgA defi ciency The serum gammaglobulin was ele- vated in eight patients out of 11 PBI and radio iodide tests were of little value in evaluating the functional state Clinical examination BEI growth curve and urinary hydroxyproline were the most useful for this Thyroid antibody titres varied considerably during the course of the disease and were significantly elevated in 77% of patients at some time or other The treatment of choice is thyroid hormone in full replacement dose probably for life

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Acta Pædiat Scand 61 63–72 1972

AN EPIDEMIOLOGICAL STUDY OF CHILD HEALTH AND NUTRITION IN A NORTHERN SWEDISH COUNTY

IV *Haematological Investigations especially in regard to Iron Deficiency Anaemia*

GOSTA SAMUELSON and STIG SJOLIN[1]

From the Department of Paediatrics University Hospital Umeå Sweden

The major section of this study was conducted in the autumn of 1967 as part of a nutritional survey of children of different ages in the county of Vasterbotten in northern Sweden (14) The specific aim of the haematological investigation was threefold First to reveal the prevalence in this area of haematological diseases in the child population secondly to investigate the possible relationship between iron intake and iron deficiency anaemia and thirdly to obtain information about the distribution of haemoglobin concentration packed red cell volume and mean red cell haemoglobin concentration in healthy children

MATERIAL

All 1401 children in the general nutritional study were included in the haematological investigation This material consisted of three main groups of children aged 4 8 and 13 years respectively in 1967 To these groups was added a fourth consisting of 80 one year old children who were not included in the general survey All children enrolled lived in one of three areas in Vasterbotten which differ from one another both geographically and in part, socio-economically (Fig 1) The detailed composition and distribution of the total material is shown in Table 1 For further details see Samuelson (14)

All the urban children were from *the city of Umeå* near the coast of the Gulf of Bothnia (Fig 1) The children belonging to the age groups 4 8 and 13 years were chosen from the official population register Every second child in these age groups in Umeå proper was selected for the study Seven children of 605 were excluded 5 because of mental retardation and 2 because they were suffering from acute gastroenteritis. The fourth and smaller group the 1 year-old children consisted of all children who reached this age in January or February 1968 and were enrolled at three different child health centres in Umeå

The children from *the inland area* lived in the large and sparsely populated rural districts of Lycksele and Asele (Fig 1) Nearly all school children in the 8 and 13 year-old groups in these districts were studied Nine were excluded for special reasons diabetes (three) chronic infections (two) mental retardation (three) and chondrodystrophy (one) Four year-old children were not investigated in this area because of the administrative problems involved in reaching preschool children living in a large rural district No children from the *town of Lycksele* were included

All children from *the mountain foreland* lived in the extensive rural districts of Dorotea and Vilhelmina (Fig 1) About 40% of the population however lived in the two modern municipalities Dorotea and Vilhelmina about 300–400 metres above sea level Only a few lived in the *real* mountains As in the inland area, only school children were included in the study Also in the mountain foreland almost all children in the 8 and 13 year-old groups were investigated Nine were excluded for different reasons diabetes (one) acute gastroenteritis (one) blindness (one) and mental retardation (six)

METHODS

All children were subjected to a general physical examination by one of us (G S) All examinations were performed in schools or in child health centres The medical and anthropometrical examinations are described elsewhere (15)

Capillary blood samples were taken from finger pricks with the child in a sitting position Disposable

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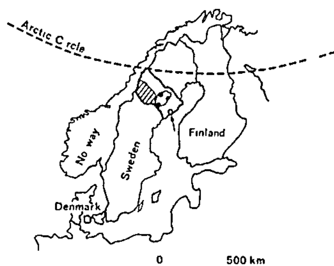


Fig 1 A map of Scandinavia showing the county of Vasterbotten and the three areas studied The arrow indicates the city of Umeå stippling shows the inland area shading the mountain foreland

needles were used and the capillary blood was allowed to drip without any compression of the finger Almost all samples were taken between 8 a m and 1 p m only exceptionally between 1 p m and 3 p m The children were not fasting Throughout the investigation all blood samples were taken by the same well trained laboratory technician

Haemoglobin concentration (Hb) was determined as cyanmethaemoglobin 0 020 ml of blood was taken in duplicate from the same finger prick using disposable pipettes (*Drummond Scientific Company USA*) calibrated for 0 020 ml ± 1° This volume of blood was mixed with 5 ml of ferricyanide potassium cyanide solution (Aculute Diluent Pellets® Ortho Diagnostics USA)

New solutions were prepared weekly and stored in the dark The readings were done in a Beckman B spectrophotometer at 540 nm The determinations were performed 2 to 10 hours after sampling i e all haemoglobin determinations were performed on the day of sampling The Beckman B instrument was calibrated daily by using a standard haemoglobin solution with an extinction coefficient of 11 5 (Acuglobin Haemoglobin Standard Ortho Diagnostics USA)

The error of a single determination was calculated from 100 duplicate determinations in each age group[1] and was found to be ±0 2 g/100 ml for all groups The mean value of 100 determinations in the age groups 4 8 and 13 years was 12 8 13 3 and 13 8 g/100 ml respectively

Packed red cell volume (PCV) was determined in duplicate on capillary blood from the finger prick with the aid of a haematocrit centrifuge (International Micro Capillary Centrifuge Model MB) Heparinized capillary tubes were used and the tubes were sealed at one end with plasticine The centrifugation time was 4-5 min and the speed 11 000 r p m The PCV was read with the aid of a special haematocrit reading chart For practical reasons the PCV readings could not be done immediately after the blood sampling but all readings were done within ? hours No correction was made for trapped plasma

The error of a single determination of PCV was calculated from 100 duplicate determinations in the age groups 4 8 and 13 years and was found to be ±0 2 ±0 4 and ±0 5 respectively The mean value for 100 determinations in the three age groups was 38 39 and 40 respectively

Blood smears were prepared in duplicate The smears were stained according to May Grunwald Giemsa for evaluation of red cells leukocytes and platelets The smears were appraised without access to the Hb and PCV values

Staining of bone marrow aspirates for iron was performed at the Department of Clinical Chemistry University Hospital Umeå by a slight modification of the method of Hansen & Weinfeld (4) Thirty children were investigated

Microsedimentation rate of erythrocytes (ESR) was determined by the method of Strom (17)

Transferrin concentration was determined on serum from capillary blood samples The specimens were taken from finger pricks in heparinized capillary tubes and centrifuged in an International Micro Capillary Centrifuge The specimens were then frozen (−20 C) until analysed The transferrin determinations were performed at the Department of Clinical Chemistry General Hospital Malmo according to an immunochemical method described by Laurell (7)

[1] According to $\sigma = \pm\sqrt{\Sigma d^2/2n}$ where d is the difference between duplicate determinations and n the number of differences

Table 1 *Age sex and geographical distribution of the 1481 children studied*

Age group Median age	1 year 1 year			4 years 4 years 5 months			8 years 8 years 4 months			13 years 13 years 4 months		
	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total	Boys	Girls	Total
City of Umeå	40	40	80	99	99	198	100	100	200	100	100	200
Inland area	—	—	—	—	—	—	98	90	188	91	111	202
Mountain foreland	—	—	—	—	—	—	99	96	195	114	104	218
Total	40	40	80	99	99	198	297	286	583	305	315	620

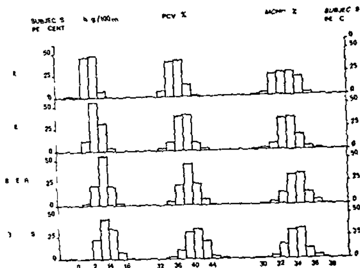


Fig 2 Distribution of Hb PCV and MCHC in the total material in relation to age

Serum iron determinations were performed at th Departm nt of Clinical Chemistry University Hospital Umeå by the method described by Agner (1)

Serum haptoglobin was determined at the Department of Clinical Chemistry University Hospital Umeå according to the method describ d by Taruko ki (18)

Haemoglobin electrophoresis was performed at the Department of Clinical Chemistry University Hospital Uppsala according to the method described by Kunkel & Wall nius (6)

4 hour recall method was used to det rmine the mean nutrient content of food consumed (14)

Statistical methods used are described separately (14)

RESULTS

The medical examination showed the children s health and nutritional status to be generally good Signs of upper respiratory infections were present in 11 1 of the 4 year-old children and in 4 8 and 6 1° of the 8 and the 13 year-old children respectively (15)

Hb PCV and MCHC (mean red cell haemoglobin concentration)

The results for the material as a whole are presented in Table 2 and Fig 2

Three children had definitely subnormal Hb PCV and MCHC (Table 3) A detailed investigation at the D partment of Paediatrics in Umeå within a month after the first investigation revealed that these 3 children suffered from iron deficiency anaemia The dietary history of case 24 the 1 year-old boy revealed that his consumption of iron rich or iron-enriched foods was very low and his iron intake was probably lower th n 4 mg a day No bleeding was demonstrated The 2 13 year old girls had both been menstruating regularly for 1 1/ years when they were examined They both menstruated during the hospital stay and the blood loss was measured during p rt of the time Case 1 253 lost 100 ml during the last 4 days of the 5-day period and case 1 292 45 ml during the last 3 days of the 5-day period The dietary histories of these two girls did not show any remarkable deviations from normal

Blood smears

Evaluation of the blood smears showed distinct hypochromasia in the red cells of the same 3 children cases 24 1 253 and 1 292 who had low Hb PCV and MCHC In these cases anisocytosis was also obvious and a few target cells were seen In 9 other children single target cells were found but no other abnormalities

Leucocytes and platelets were judged to be roughly normal in all cases In some cases neutrophil leukocytosis or lymphocytosis was present but no counts were done

Table 2 *Mean values and S D of Hb, PCV and MCHC in the total material of 1481 children*

Age group		1 year		4 years		8 years		13 years	
No of subjects		80		198		583		620	
		Mean	S D	Mean	S D	Mean	S D	Mean	S D
Hb g/100 ml	Boys	12 12	0 79	12 74	0 64	13 45	0 67	14 05	0 86
	Girls	12 14	0 61	12 84	0 68	13 66	0 72	13 78	0 82
	Total	12 13	0 70	12 79	0 66	13 55	0 70	13 91	0 85
PCV	Boys	36 8	1 76	38 4	1 83	39 5	1 73	41 3	2 38
	Girls	37 0	1 72	38 7	1 87	39 9	1 94	40 6	2 21
	Total	36 9	1 73	38 5	1 85	39 7	1 85	41 0	2 32
MCHC	Boys	33 0	1 60	33 2	0 98	34 1	1 13	34 0	1 21
	Girls	32 8	1 20	33 2	1 12	34 2	1 26	33 9	1 13
	Total	32 9	1 41	33 2	1 05	34 1	1 20	34 0	1 17

ESR

The results are published in a previous article (15)

Transferrin

The results are presented in Table 4 The mean transferrin values were somewhat lower than the values found by Högberg (2) Two children with iron deficiency anaemia had elevated values (Table 3) No significant differences were found between the three areas

Special haematological investigations in a selected group of children

All children with border line or low Hb and/or PCV values i e those who had Hb and/or PCV at or below the mean—2 S D were subjected to a more detailed haematological investigation All 30 children who met this criterion were examined at the Department of Paediatrics in Umea 2 weeks to 5 months after the first investigation The examination included determination of serum iron serum transferrin concentration serum haptoglobin, staining of bone marrow aspirates for iron and in 17 cases also haemoglobin electrophoresis In the 3 children already mentioned (Table 3) iron deficiency was proved to be the cause of the low Hb and PCV whereas in the other 27 children no haematological disease could be incriminated as the cause of the low Hb and/or PCV All 27 of these children had stainable iron in their bone marrow

Comparison between the age groups

The differences in mean Hb and PCV between the four age groups were statistically significant throughout (1 vs 4 years $p < 0\ 001$ 4 vs 8 years $p < 0\ 001$ 8 vs 13 years $p < 0\ 001$)

Comparison between boys and girls

There was no significant difference in mean Hb and PCV between boys and girls in the 1 and 4-year old groups (Table 2) In the 8 year old group the girls had a higher mean Hb ($p < 0\ 001$) and PCV ($p < 0\ 01$) than the boys

Table 3 *Children with definitely subnormal Hb PCV and MCHC*

Case no	Sex	Area	Age group	Hb (g/100 ml)	PCV ( )	MCHC ( )	Red cells in blood smear	Bone marrow iron	Transferrin μg/100 ml	Effect of iron
24	Boy	City of Umeå	1 year	9 4	36	26	Hypochromic	0	—	+ + +
1253	Girl	Mountain foreland	13 years	9 9	33	30	Hypochromic	0	437	+ + +
1292	Girl		13 years	8 8	31	28	Hypochromic	0	476	+ + +

Table 4 *Means and S D of transferrin in serum of 1 4 8 and 13 year old children The values are given in ug per 100 ml*

	1 year			4 years			8 years			13 years		
	No	Mean	S D	No	Mean	S D	No	Mean	S D	No	Mean	S D
Boys	18	331	29 1	83	331	46 2	216	318	36 6	238	337	46 3
Girls	23	327	31 6	81	3 0	46 4	231	3 3	39 6	256	335	4 8
Total	41	3 8	30 2	164	326	46 1	447	3 1	38 2	494	336	44 5

The 13 year old boys had significantly higher Hb ($p < 0\,001$) and PCV ($p < 0\,001$) than the girls

Comparison between children from different geographical areas

A comparison between children from the three different geographical areas in regard to Hb and PCV was possible only for the age groups 8 and 13 years (Table 5)

All differences are rather small Above all there was a tendency for children from the city of Umeå to have lower Hb and PCV than children from the other two areas This tendency was most obvious in 8 year-old boys and 13 year old girls (Table 5)

Iron intake in relation to Hb concentration

In all 1 401 children belonging to the age groups 4 8 and 13 years the iron intake was calculated from a food consumption study by the 24 hour recall method (14) The mean and

Table 5 *t Tests on the difference of the means of Hb and PCV in different age groups and geographic areas*

1 City of Umeå 2 = Inland area 3 = *Mountain foreland*
n s = not significant

	Geographic areas	Hb Difference (g 100 ml)	Hb p	PCV Difference ( )	PCV p
8 year-olds					
Boys	1 vs	−0 23	<0 05	−0 82	<0 001
	1 vs 3	−0 21	<0 05	−0 83	<0 01
	2 vs 3	−0 0	n s	0 01	n s
Girls	1 vs 2	−0 03	n s	+0 07	n s
	1 vs 3	0 13	n s	−0 19	n s
	2 vs 3	−0 16	n s	−0 1	n s
Total	1 vs 2	−0 10	n s	−0 40	<0 05
	1 vs 3	−0 17	<0 05	−0 51	<0 01
	2 vs 3	−0 07	n s	−0 11	n s
13 year-olds					
Boys	1 vs 2	+0 19	n s	+0 52	n s
	1 vs 3	−0 11	n s	−0 2	n s
	vs 3	−0 30	<0 05	−0 74	<0 05
Girls	1 vs 2	−0 9	<0 01	−0 93	<0 01
	1 vs 3	0 25	<0 05	−0 76	<0 05
	2 vs 3	+0 04	n s	+0 17	n s
Total	1 vs 2	0 05	n s	0 21	n s
	1 vs 3	−0 19	<0 05	−0 51	<0 05
	2 vs 3	−0 14	n s	−0 30	n s

Table 6 *Mean iron intake in mg according to a 24 hour recall food consumption study in 1401 children aged 4 8 and 13 years*

Median values are given within parentheses

Age group	4 years		8 years		13 years	
	Boys	Girls	Boys	Girls	Boys	Girls
City of Umeå	11 1 (10 5)	9 8 (9 0)	13 4 (11 9)	11 5 (10 4)	14 0 (13 2)	11 6 (11 4)
Inland area			14 5 (13 6)	11 2 (10 0)	16 9 (14 8)	13 9 (12 6)
Mountain foreland			15 6 (14 0)	13 2 (12 0)	19 0 (17 4)	14 5 (12 9)
Total	11 1 (10 5)	9 8 (9 0)	14 6 (13 3)	12 0 (10 7)	16 7 (15 0)	13 5 (12 4)

median iron intakes are presented in Table 6 The iron intake increased with age boys had a higher intake than girls and the highest intake in each age group was observed in the mountain foreland In all groups the intake varied within wide ranges

In order to find out whether a relationship existed between a low iron intake as measured by the 24 hour recall method and a low Hb the Hb distribution of 8 and 13 year old children with iron intakes of less than 8 mg was compared with the Hb distribution of the other 8- and 13 year-old children No difference was found Among the 149 with a low daily mean iron intake (≤8 mg), 18 0% had a Hb≤13 0 g/100 ml, of the other 1 054 with an iron intake above 8 mg per day 15 8% had a Hb≤ 13 0 g/100 ml

DISCUSSION

Three children of 1 481 investigated i e 0 2%, had overt iron deficiency anaemia i e in the age group 1 year, the incidence was 1 3%, and among the 13 year old girls the incidence was 0 6% Since the child material included in the study was a randomized and large sample of the total child population in these ages the incidence figures found also signify the prevalence of iron deficiency anaemia in these communities That no other blood diseases were diagnosed probably means that haematological disorders other than iron deficiency anaemia are relatively rare in these age groups

The low incidence of iron deficiency anaemia in these districts is in contrast to the findings in many foreign countries especially among children from low socio economic backgrounds

Table 7 *Scandinavian investigations on the Hb concentration (g/100 ml) in children aged 1, 4, 8 and 13 years*

		1 year			4 years			8 years			13 years		
		No	Mean	S D	No	Mean	S D	No	Mean	S D	No	Mean	S D
Odin (1934)	Boys	56	9 1	—	49	10 1	—	288	10 6	—	215	11 2	—
	Girls	29	9 2	—	39	10 4	—	285	10 5	—	228	11 0	—
Vahlquist (1941)	Boys	22[a]	12 23	1 04	—	—	—	31[b]	12 24	0 95	25[c]	13 64	1 33
	Girls				—	—	—	30[b]	12 64	0 76	23[c]	12 64	0 84
Moe (1963 1965)		135	11 92	0 57	—	—	—	—	—	—	—	—	—
Marner (1969)		—	—	—	38	13 5	0 79	—	—	—	—	—	—
Natvig (1969)	Boys	—	—	—	—	—	—	28	13 00	0 73	34	13 36	0 94
	Girls	—	—	—	—	—	—	22	12 93	0 78	37	13 31	0 84
Samuelson & Sjolin (1971)	Boys	40	12 12	0 79	99	12 74	0 64	297	13 45	0 67	305	14 05	0 86
	Girls	40	12 14	0 61	99	12 84	0 68	286	13 66	0 72	315	13 78	0 82

[a] 1 to 1¹/₂ years old [b] 7 years old [c] 14–15 years old

Table 8 *Percentage of 13 year old girls who attained menarche at different ages in the three areas*

	Age in years					Total % of menstruating 13-year-old girls
	9	10	11	12	13	
City of Umeå	1 0	5 0	11 0	27 0	22 0	66
Inland area	0	0	6 3	23 4	31 5	61
Mountain foreland	1 0	2 9	9 6	25 0	26 9	66

(13) and to the findings of a study carried out in the two northernmost counties in Sweden Vasterbotten and Norrbotten by Odin (12) about 40 years ago The incidence of iron deficiency anaemia is not given in Odin s study but a comparison of the mean Hb found by Odin with that found in our study (Table 7) clearly shows that anaemia (probably in most cases iron deficiency anaemia) must have been much more common in this part of Sweden at that time The differences may to some extent be due to methodological differences in the determination of haemoglobin However the improved situation is no doubt mainly due to generally improved socio-economic conditions with easier a cess to better food and to better health services for children including systematic iron prophylaxis for all low birth weight infants and a widespread use of iron enriched formulas in infant f eding The relationship between child health food habits and socio-economic conditions is discussed elsewhere (16)

It is characteristic that the only child who had acquired iron deficiency anaemia by a deficient iron intake belonged to the 1 year old group In infants and small children the risk of iron deficiency anaemia can be minimized only by the adoption of large scale and special pre cautions against a suboptimal iron intake

That blood loss through menstruation often causes iron deficiency anaemia is well known Hallberg et al (3) have shown that the median blood loss is about 30 ml per period in 15 year old girls and also that a negative iron balance occurs in women at losses of more than 60 ml Both our 13 year-old girls with iron deficiency anaemia had had regular menstruations for a long time when examined and at a control in hospital it was shown that they lost more than this critical amount of blood during a menstrual period It therefore seems likely that their anaemia was caused by excessive menstrual blood losses Since the age of menarche is steadily decreasing iron deficiency anaemia caused by heavy menstrual bleeding is becoming more and more a problem for paediatricians (20 21)

The well-documented fact that Hb and PCV increased with age was clearly confirmed in this study

A difference in Hb and PCV between the sexes was demonstrated in the age group 13 years in which the boys as expected had significantly higher values than the girls A negative iron balance due to increased iron losses in some menstruating girls and the effect of increased androgen production on haemoglobin formation in pubescent boys are probably the main factors responsible for this difference That Hb and PCV were slightly higher in 8 year old girls than in 8 year-old boys is difficult to explain On an average the boys consumed more not less iron than the girls (Table 6) The incidence of infections immediately before and at the time of examination was the same for boys and girls and there was no difference in the distribution of ESR in the two groups No significant difference in height or weight was observed between boys and girls We are thus unable to explain the small differences in Hb and PCV between 8 year-old boys and girls Vahlquist (19) however found a similar difference (Table 7)

The finding that children from the city of Umea tended to have lower Hb and PCV than children from the inland and the mountain fore land deserves special attention. Odin (12) found

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Mountain foreland			15 6 (14 0)	13 2 (12 0)	19 0 (17 4)	14 5 (12 9)
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In order to find out whether a relationship existed between a low iron intake as measured by the 24 hour recall method and a low Hb the Hb distribution of 8 and 13-year old children with iron intakes of less than 8 mg was compared with the Hb distribution of the other 8 and 13 year old children No difference was found Among the 149 with a low daily mean iron intake (≤ 8 mg) 18 0% had a Hb ≤ 13 0 g/100 ml of the other 1 054 with an iron intake above 8 mg per day 15 8% had a Hb ≤ 13 0 g/100 ml

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[a] 1 to $1^1/_2$ years old [b] 7 years old [c] 14–15 years old

SUMMARY

As part of an epidemiological study of child health and nutrition in a northern Swedish county haematological investigation of 1 481 healthy children aged 1 4 8 and 13 years was carried out This consisted of Hb and PCV determinations and examination of blood smears Overt iron deficiency anaemia was found in three children one 1 year old boy and two 13 year-old girls The prevalence of iron deficiency anaemia among 4 8 and 13 year-old children taken together was 0 1% Among the 13 year-old girls it was 0 6 No oth r blood diseases were diagnosed

More detailed haematological investigation of the 27 apparently healthy children found to have low Hb and/or PCV (≤the mean −2 S D) did not reveal any underlying haemato logical disease A higher incidence of recent or subclinical infections in this group than in the material as a whole suggests that at least some of these 27 children had low Hb and or PCV as a result of infections

Compared with findings 40 years ago the m an Hb conc ntration had increased by 2 4 to 3 1 g 100 ml in the different age groups This change was regarded as a result of im p oved socio economic living conditions includ ing better health services for children

ACKNOWLEDGEMENTS

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W tha k Mr Greta Moren for skilful te hnical a s ce and Miss E a Thulin who p rformed the tra ferrin naly is

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a similar but much more pronounced difference in Hb between school children from the coastal area and those from the inland and mountains The mean difference was 0 99 g/100 ml for boys and 1 19 g/100 ml for girls, and for both sexes the difference was statistically significant Odin pointed out that the difference in altitude above sea level could explain only a small part of the difference He was not able to prove that differences in food composition played a role but believed that genetic factors due to the presence of Lapps in the population could be relevant However, to our knowledge real evidence indicating that genetic factors influence the Hb level has never been presented

In our material the average intake of iron increases from coast to inland to mountain foreland in all age groups studied (Table 6) and it is tempting to regard this as a possible cause of the differences found in Hb and PCV However there is no close parallel between average iron intake and the Hb values in the different geographic groups Furthermore the differences in Hb and PCV are statistically significant only for 8 year old boys and 13 year old girls (Table 5) Differences in altitude even if small might be the main explanation of the small geographic differences found The fact that the difference in Hb between coast and inland has decreased so much during the last 40 years must mean that environmental factors of some kind caused the great difference in Hb 40 years ago

For several obvious reasons it is of interest to compare our results with those of others In Table 7 comparable Scandinavian investigations are presented (8 9 10 11 12 19) It is evident that recent studies have given similar results

Whether the results of our study can be regarded as representative for a normal healthy child population and serve as reference values must be considered All children included were selected at random and the study samples were doubtless large enough to be representative of all children in the respective age groups in the three geographic areas In addition to age sex and altitude the intake of iron the presence of diseases, especially infections (5) and blood losses can influence the Hb concentration in man Some of these factors are extremely difficult to control, particularly in a large field study like ours and their relative contributions to the results are difficult to evaluate The 24 hour recall showed that the average iron intake for the three age groups (4 8 and 13 years) was fairly satisfactory in relation to recommended allowances and only two children had overt iron deficiency anaemia, but on the other hand it has been shown that the addition of iron to food can cause a significant rise in the mean Hb of healthy infants (9) The influence of menstrual blood loss is also very difficult to estimate In our material a considerable number of girls were menstruating at the age of 13 years (Table 8) and many might have had a negative iron balance without any clinical signs of iron deficiency Furthermore the investigation revealed a rather high incidence of minor infections immediately before or at the time of the haematological examination Among the 30 children with low Hb and PCV only 3 had iron deficiency Of the other 27 children 8 had signs of acute upper respiratory infection at the time of examination or had had such an infection during the preceding week One child had a silent urinary tract infection Furthermore 48% of the 27 children had an ESR higher than 20 mm whereas in the total material the corresponding incidence of increased ESR was 20 %

This leads to the conclusion that in a certain number of children the Hb and PCV values obtained were not optimal Obviously this statement applies to most materials of normal Hb and PCV hitherto published As a rule neither menstruation nor infection has been taken into account Infections in particular are important to consider during childhood In spite of these objections it can still be argued that the results as presented in Table 2 ought to be quite useful in practical clinical work if only they are used in a sensible way i e with due regard to the fact that the optimal Hb and PCV values are probably somewhat higher

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THE POSSIBILITY OF MATERNO FOETAL TRANSFER OF LYMPHOCYTES IN MAN

LARS OLDING

From the Institute of Pathology University of Uppsala Uppsala Sweden

Investigations dealing with the possible trans placental transfer of leucocytes from the mother to her offspring have been few and have given contradictory results Benirschke et al (1 2 3) have recently made comprehensive reviews of studies on materno-foetal chimerism of lymphocytes and these reports will therefore be only briefly touched upon here

Desai & Creger (4) using maternal leucocytes labelled with atabrine showed fluorescent cells in cord blood from 6 out of 9 newborn infants Not only leucocytes were found but also platelets and multinuclear cells (probably trophoblasts) Turner et al (8) however found occasional lymphocytes with a female karyotype in cord blood from only 2 out of 183 newborn boys and they examined 30 cells in every case Benirschke & Sullivan (3) observed a small number of cells with an XX karyotype in blood collected from the foetal vessels of the delivered placenta belonging to 3 out of 4 examined newborn boys However the authors found no female cells in blood from these boys 6 weeks after delivery They examined about 30 metaphases in every case

In the investigation reported below the possibility of transplacental transfer of lymphocytes was studied using sex chromosomes as a marker umbilical cord blood of newborn male infants was examined for the presence of lymphocytes with a female karyotype A large number of cells was analysed in every case No interference was introduced until the blood was sampled

MATERIAL AND METHODS

The series consisted of 14 male infants all singletons 10–15 ml of blood was collected in tubes after careful cleaning of the cord and a few drops of heparin were added The blood was withdrawn immediately after delivery of the child but before expulsion of the placenta

Separation of the leucocyte-containing plasma was accelerated by adding 1 part Macrodex to 5 parts blood Buffy coat cultures were prepared and harvested in all essentials according to Moorhead et al (6) As a medium a mixture of 70% Tc 199 (Difco) and 30% autologous plasma from the cord blood was used All metaphases of acceptable technical quality were photomicrographed After development the negative film was projected in a microfilm reader whereby the chromosomes could be easily studied again on the screen of the reader In all doubtful cases prints were made and the karyotypes of the photographed chromosomes were constructed and they were compared with the original metaphases in the microscope Only metaphases including 46 positively identified chromosomes were included in the results

In all cases except one the placenta was examined morphologically Several specimens from the umbilical cord the foetal membranes and the placenta were taken for microscopical examination

RESULTS

Because of the variation of both the mitotic index and the technical quality the number of available metaphases varied considerably in the different cases (Table 1) None of the 14 cases representing altogether 1 772 examined

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MATERIAL AND METHODS

The series consisted of 14 male infants all singletons 10–15 ml of blood was collected in tubes after careful cleaning of the cord and a few drops of heparin were added The blood was withdrawn immediately after delivery of the child but before expulsion of the placenta

Separation of the leucocyte-containing plasma was accelerated by adding 1 part Macrodex to 5 parts blood Buffy coat cultures were prepared and harvested in all essentials according to Moorhead et al (6) As a medium a mixture of 70% Tc 199 (Difco) and 30% autologous plasma from the cord blood was used All metaphases of acceptable technical quality were photomicrographed After development the negative film was projected in a microfilm reader whereby the chromosomes could be easily studied again on the screen of the reader In all doubtful cases prints were made and the karyotypes of the photographed chromosomes were constructed and they were compared with the original metaphases in the microscope Only metaphases including 46 positively identified chromosomes were included in the results

In all cases except one the placenta was examined morphologically Several specimens from the umbilical cord the foetal membranes and the placenta were taken for microscopical examination

RESULTS

Because of the variation of both the mitotic index and the technical quality the number of available metaphases varied considerably in the different cases (Table 1) None of the 14 cases representing altogether 1 772 examined

Table 1. *Results of the study on materno-foetal passage of lymphocytes indicated by cells with female karyotype in the cord blood of newborn boys*

Case no.	Cord blood: No. of cells analysed	Cord blood: No. of cells with female karyotype	Pregnancy and delivery	Morphology of the placenta
1	102	0	Normal	Not examined
2	73	0	*Toxemia of pregn.* + prolonged labour	*Only one umbilical* artery
3	28	0	Normal	Normal
4	36	0	Normal	Placentitis
5	177	0	Normal	*Large infarction*
6	135	0	Normal	Normal
7	149	0	Normal	Normal
8	37	0	Normal	Normal
9	162	0	Normal	Normal
10	203	0	Rh sensitization	Normal
11	233	0	Normal	Normal
12	199	0	Protracted pregn.	Normal
13	131	0	Premature rupt. of membranes	Normal
14	108	0	Normal	Normal
Total	1 772	0		

cells showed any cell with a karyotype compatible with a female one.

In a previous preliminary communication (7) concerning the results of examination of 753 cells from 8 newborns, which was the total up to that time, I reported a finding of altogether 3 cells of presumably maternal origin in the cord blood of 2 boys. Since then the karyotype of these three cells have been kindly reviewed also by Professor Albert Levan at the Institute of Genetics at the University of Lund, Sweden, and it is now clear that these karyotypes are more compatible with male than female ones. Thus my preliminary report has to be corrected on this point.

No chromosome abnormalities were discovered in the 14 cases, and the infants were not malformed.

The pregnancy and delivery were normal in 10 of the 14 cases; in 2 cases there was a history of toxemia or Rh sensitization, and protracted delivery or premature rupture of membranes was noted in 2 cases. The morphological examination of the placenta revealed abnormalities in 3 cases; in one of them placentitis was shown, in 1 case there was only one artery, and in one a large infarction.

DISCUSSION

It is obvious from this investigation that maternal viable lymphocytes capable of division appear in the blood of the newborn infant very seldom, if ever, in normal single births. The present results are at variance with the findings of Desai & Creger (4) quoted above. However, the fluorescent method used by these authors does not seem to be entirely satisfactory, as reutilization of the atabrine labelled marker is difficult to exclude, and the labelling may also damage the cells.

The results of the present investigation tally well with those reported by Turner et al. (8), mentioned briefly above. In their studies the only two newborn boys (out of 183 examined) in whose cord blood occasional female cells were disclosed were malformed and they died shortly after delivery. In addition one of the two boys had cells with an XO karyotype pattern, and the authors suggested that lymphocytes with the ability to proliferate cross the placenta only when the foetus and the placenta are developmentally defective. It must be emphasized, however, that the absence of maternal cells in the cord blood does not exclude entirely the possibility of occasional mat-

ernal cells crossing the placenta It is feasible that some maternal lymphocytes actually do pass through but are rapidly removed from the foetal circulation The findings of Benirschke & Sullivan (3) of the occurrence of some lymphocytes with a female karyotype in the blood of the foetal vessels of the delivered placenta but no detectable maternal cells in the infants blood after delivery support this theory It seems also conceivable that a leakage of cells could take place through a damaged site somewhere in the large villous surface of the human placenta which at the end of gestation has an area of 11–14 m and a thickness of only a few microns

Finally it may be argued from a theoretical standpoint that absence of maternal lymphocytes or a finding of only occasional such cells in the foetal blood may be due to different patterns of behaviour of the maternal and foetal cells during the culture for the chromosome preparation e g different optimal incubation times for division or some interaction between the maternal and foetal cells in the culture This problem was not studied in the present investigation

The importance of a conceivable materno-foetal chimerism of lymphocytes cannot be assessed Kadowski et al (5) described a 16 month-old boy with a congenital deficiency syndrome including thymic alymphoplasia and with symptoms suggestive of the runting syndrome apparently due to prenatal transfer of maternal lymphocytes to the foetus (proven by chromosome studies) It is not known however whether maternal lymphocytes can survive in normal infants

SUMMARY

The occurrence of maternal lymphocytes in the cord blood of the recently delivered infant was studied Cells with a female karyotype were searched for in the cord blood of 14 newborn boys all singletons The blood was collected before expulsion of the placenta The whole investigation comprised analysis of 1 772 cells In none of the 14 boys were any female cells discovered It may be concluded from the results that maternal lymphocytes very seldom or never cross the placenta in normal deliveries or that occasionally transferred maternal cells are rapidly removed from the foetal circulation

ACKNOWLEDGEMENTS

Thanks are due to Mrs Karin Byman and the midwives at the Obstetrical Department in Uppsala for skilful technical assistance

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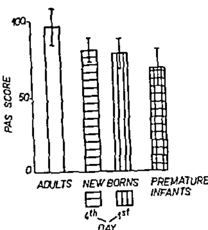


Fig 1 Mean values and standard deviations of platel t glycogen score in adults full term newborns and premature infants

had been induced by medication the others had a normal birth All the premature babies were examined on the first day of life (17 babies) or on the second day of life (3 babies) The clinical condition cor responded to the stage of prematurity a complication showing an early asphyxia syndrome of mild grade appeared in 2 infants 11 children were administered vitamin K and adenosine triphosphate one baby received alcalisation treatment before it was ex amined

Results of the individual groups of newborn infants were compared with a control group of 25 normal adult subjects The t test was u ed to determine the significance of differences betw en mean values The correlation coefficient was employed to analyse the relationship between platelet glycogen score and birth weight of premature infants and gestational age

RESULTS

The mean values of the platelet glycogen score and the standard deviation (S D) of all ex amined groups are shown in Fig 1

The mean values of all three groups of new born infants are lower than in the control group of adults with mean value 96 ± 12 (S D) In the group of 4-day old infants the average score amounts to 80 4 ± 8 2 (S D) in the group of 1-day-old full term babies the score amounts to 76 7 ± 10 2 (S D) The lowest score was found in the group of premature infants 67 8 ± 12 9 (S D)

The differences in the mean values of all groups of newborn infants are statistically highly significant compared with the values of the control group for 1-day-old infants (t = 5 67 $p < 0\ 001$) for 4-day-old infants (t = 7 59 $p < 0\ 001$) for premature infants (t = 7 59 $p < 0\ 001$) If we compare the individual groups of infants we find a highly significant difference between the premature infants and the 4-day-old full term newborn infants (t = 3 72 $p < 0\ 001$) The difference between the premature infants and the 1-day-old full term infants is also significant (t = 2 46 $p < 0\ 02 > 0\ 01$) The difference between 1-day-old and 4 day-old full term infants is not significant (t = 1 23 $p < 0\ 1$)

We also determined the relationship between the platelet glycogen score and the birth weight of premature infants (Fig 2) There

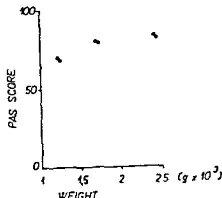


Fig 2 Correlation between platelet glycogen score and birth weight of premature infants

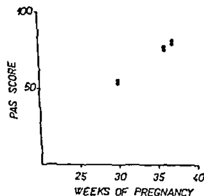


Fig 3 Correlation between platelet glycogen score and gestational age of premature infants

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GLYCOGEN CONTENT IN BLOOD PLATELETS IN NEWBORN INFANTS

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In our previous studies we have already pointed out certain special physiological properties of infants blood platelets during the first days of life (13, 10 11 14 12) The functional changes observed included defective platelet adhesiveness and aggregation when exposed to glass retardation in the course of viscous metamorphosis in observation by phase contrast microscopy, hypoaggregability in the presence of adenosine diphosphate adrenalin and noradrenalin and decreased platelet factor 3 activity as well as impaired platelet factor 3 release Premature babies showed particularly marked alterations Apart from these phenomena, the maximum amplitude in thrombelastogram curves was diminished during the first 2 days of life Mull & Hathaway (17) also proved a defect in platelet aggregation to collagen and thrombin as well as defective clot retraction The above described alterations resemble defective platelet functions seen in thrombasthenia They are of transitory character and reflect developmental changes which have been found in many other systems of the newborn organism (2) In an attempt to find metabolic differences in these platelets we attempted to determine the glycogen content in the platelets of full term infants and premature babies, using a cytochemical scoring method enabling us to perform our examinations even on a small amount of blood

MATERIAL AND METHOD

The stainable glycogen in blood platelets was evaluated by using the cytochemical scoring method previously described (7 8) The venous blood of the newborn babies was taken from the cranial or cubital vein with 23 gauge needles into plastic test tubes containing an hypercitrated acid-citrate dextros solution (0 126 M sodium citrate 0 13 M citric acid 2% dextrose) and mixed in the proportion 9 1 The entire citrated blood amount needed for testing was 4 ml The blood has to be processed within 2 hours of drawing at the latest and is kept in a refrigerator at a temperature of +4 C till use Platelet rich plasma was gained by slow centrifugation at room temperature (1 000 r p m for 10 min) The smears prepared from the platelet suspension were stained for glycogen by the PAS procedure and observed in phase contrast microscope with blue filter Under these conditions the positive platelets show brilliant bluish greenish granules varying in size and number According to the amount of stainable glycogen they were divided in four groups from 0 to 3 One hundred platelets were scored in every examined smear and the total score for one hundred platelets indicated the mean amount of stainable glycog n in the blood sample

Three groups of newborn infants were examined with this method

1) A group of 20 full term newborns in the age up to 24 hours of life They were from normal pregnancy and delivery and had normal birth weight

2) A group of 20 normal full term infants on the 4th day of life

3) A group of 20 premature babies with 1 210 to 2 460 g birth weight (5 of them between 1 210 g and 1 500 g 6 of them between 1 500 and 2 000 g 9 of them between 2 000 and 2 460 g) The infants of this group were born between the 26th and 40th week of pregnancy (8 of them to the 32nd week 10 of them between the 33rd and 37th week and 2 after the 37th week) Two of these infants were delivered by Caesarean operation one of them after the d livery

cially respiratory distress syndrome nutrition and cold exposure Some of these circum stances could explain the insufficient correla tion between the platelet glycogen and the birth weight as well as the gestation age They also require a dynamic study of the platelet glycogen in hourly intervals after birth In the skeletal muscles of the newborn infant the glycogen amount is three to five times larger in the heart muscle about 10 times higher than normal values in adults and decreases to reach these values in the course of a few days after birth (22) Moreover some authors (20) found in experimental animals that glycogen of foe tal liver was different in the infrared absorption spectra and suggested that a deficiency of 1 4 to 1 6 transglucosidase (branching enzyme) might exist at this time

The activation of platelet glycogenolysis by various agents could contribute to the ex planation of some questions of glycogen metabolism in newborn s blood platelets This is a subject of our future work

SUMMARY

The stainable glycogen in blood platelets was evaluated in 60 newborn infants using cyto chemical scoring method 20 full term new borns in 24 hours after birth 20 full term new borns on the 4th day of life and 20 premature infants on the first or second day of life were studied Mean values of all three groups were significantly lower than the mean of a control adults group The mean glycogen content of premature infants was also significantly lower than the mean glycogen content of the 4 day old full term newborns and of the 1-day-old full term newborns No correlation was found between the platelet glycogen and the birth weight as well as between the platelet glycogen and the gestational age in premature babies

The reduced glycogen content in newborn s platelets seems to reflect either a high ex haustion of glycogen stores or an insufficiency in the synthesis of this reserve substance in blood platelets

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is no correlation between these two values if we evaluate the entire examined group

The relationship between the platelet glycogen score and the week of the gestational age of premature infants was also determined Even here however, no convincing correlation was found for these two values The correlation coefficient ($r = 0\ 4$) did not reach a significant level

DISCUSSION

Typical of blood platelets is a high metabolism intended to supply sufficient energy for the basic functions of blood platelets in the hemostasis and hemocoagulation It was found that viscous metamorphosis and retraction are processes which depend strongly on the energy potential of platelets (3 16 4) The main and immediate energy source is presented by the energy rich phosphates especially adenosine triphosphate and adenosine diphosphate Glycolysis and respiration are the main factors in the metabolic processes needed for their synthesis The reserve substance for these processes is glycogen Its content in normal platelets is high (28 ± 10 mol/10^{11} platelets (3) $101\ 1 \pm 11\ 1$ g/10^{1} platelets (19)) During the last few years it has become a subject of interest for biochemical (3 23) electron microscopical (15) and cytochemical (9 5 6) studies Although the glycogen granula do not have any specific intracellular localisation variations in the glycogen content of platelets were found in pathological thrombocytes (15) The same applies to the retraction dependence on glycogen reserves (19) and glycogen splitting (23)

The cytochemical estimation of the glycogen content using a suitable scoring technique enabled us to evaluate the platelet glycogen in small infants where biochemical quantitative determination would not be possible because of large amount of blood needed for such examination In newborn infants, the biochemical properties of platelets are a special field of interest because of the multiple functional alterations of blood platelets as well as the known evolutionary changes in metabolism and enzymes of other components of the newborn s blood

Data on the amount of glycogen in platelets of newborn infants have not been previously reported The significant diminution of stainable glycogen in blood platelets of full term but especially premature infants provides a first information on the reduced content of this substance in comparison to adults The real glycogen content determined by a biochemical method would be probably even lower if we only take into consideration how small and fine the stainable glycogen granules are in comparison to thrombocytes of adult subjects Another difference of newborn s platelets was demonstrated by Barthelmai (1) He studied 29 enzymes of the glycolytic chain pentose phosphate cycle transamidasis citrate cycle and splitting of energy rich phosphates Enzymatic changes found in his study cannot be brought into direct relation to the glycogen content

The reason for the glycogen reduction in the platelets of newborn infants remains unsolved It can reflect either a high exhaustion of glycogen for the energy needs of platelets or an insufficiency in the synthesis as presumed for the glycogen in foetal liver skeletal muscles and heart (18) Apart from that we also have to consider whether the platelet glycogen is not subjected to similar dynamics as the glycogen in liver skeletal muscles and myocardium As with other species human foetuses show a quick increase in the liver glycogen shortly before birth i e between the 36th and 40th week of gestation (21) After birth the liver glycogen falls sharply its concentration decreases in the course of 24 hours to values of 10% of the initial value After that a slow increase starts In premature infants in which the initial concentration is lower than in full term infants the liver glycogen is nearly exhausted during 12 hours after birth Its concentration does not vary only with the age and maturity but also with other clinical signs and conditions as anoxia espe

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KETOTIC HYPOGLYCAEMIA ASSOCIATED WITH TRANSIENT BRANCHED CHAIN AMINOACIDEMIA

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Ketotic hypoglycaemia was described in detail in 1964 by Colle & Ulstrom (2) In this condition episodes of neuroglycopenia are transient and recur after long periods of freedom The patients are often of low birth weight and the onset lies between 14 months and 5 years The attacks always occur in the morning the symptoms varying from apathy to coma and convulsions During the episodes the urine contains a large amount of ketone bodies A provocation test using a ketogenic diet will induce neuroglycopenia in a positive case within 24 hours

In 1961 and 1966 Morris et al (22 23) described a Californian family and in 1964 Kiil & Rokkones (18) described a Norwegian family each with two children who intermittently showed neurological symptoms and signs of toxic encephalopathy They also exhibited biochemical changes consistent with an inability to metabolize the branched-chain keto acids during their acute episodes In contrast to the original and classical type of maple syrup urine disease MSUD (4) the onset was late and the children perfectly normal between their attacks Dancis et al (5) later demonstrated that the leucocytes from one of the children of each family possessed a greatly reduced branched-chain keto acid decarboxylase activity which is deficient in cases of MSUD (4) This syndrome has since then been called the intermittent or variant form of MSUD

In this paper we will describe a case of ketotic hypoglycaemia simulating a variant of the intermittent type of MSUD in a 6-year-old boy with severe brain damage who had been observed clinically since birth but in whom the biochemical abnormalities typical of MSUD such as raised blood levels of the branched chain amino acids were first seen in connection with an episode of lethargy with series of tonic convulsions and vomiting The case has been shortly reported earlier (14 15)

CASE REPORT

K S This 6 year old boy was born on June 4th 1964 He is the second son of healthy non related parents His eld r brother born in 1957 has developed normally

During the 7th month of pregnancy the mother was found to have constant proteinuria and a blood pressure of 190/120 mmHg Delivery occurred 3 weeks before term and was un ventful The birth weight was 1 860 g At birth the boy was flaccid with irregular respiration a weak cry and an Apgar score of 5 During the neonatal period he was in poor condition with convulsions and showed abnormal neonatal n urology with absence of the Moro reflex Massive scleredema gradually developed during the first weeks On ophthalmological examination bilateral cataract and corneal opacities were found

Repeated follow up examinations revealed that his somatic and psychomotor development were markedly delayed Motor development remained stagnant at an approximate level of 6–7 months throughout the age period of 1–3 years When 4½ years old he was microcephalic with a head circumference of 45 cm dwarfed with a height of 88 cm and thin with a

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Key words Blood platelets newborns glycogen cytochemical scoring method

X-ray Except for the skull the whole skeleton showed signs of osteoporosis This was particularly marked in the spine and the pelvis and probably du to a marked muscular inactivity according to his cerebral palsy The ossification center development was within normal limits Intravenous urography showed normal findings

Laboratory findings in free intervals Routine blood values were all normal Blood pH 7 47–7 57 Pco 30 5–36 mmHg On different occasions serum phosphate was at a level of 3 2 3 4–4 4 mg/100 ml, serum cal ium at 4 7–4 8–4 9 5 0 mEq/l Serum electrolytes were all r peatedly normal Alkaline phosphatases were 4 4 6 2 B L units total bilirubin 0 9 mg/100 ml thymol 0 3 units GOT 30 units and GPT 10 units Fastin blood sugar in free intervals 57 90 113 mg/100 ml Plasma lipids Cholesterol 155 mg/100 ml, phospholipid 199 mg/100 ml triglycerides 117 mg 100 ml

Intravenous glucose load (0 3 g glucose per kg bodyweight) showed a p rcentage disappearance per minute expressed as K_G value of 2 2 (corresponding to a half time of 32 min)

Urinary examination on repeated occasions excluded proteinuria and glycosuria The phosphat excretion was 370 mg/240 ml urine pho phate clearance was found to be 0 25–0 34 ml/min

Routine cerebrospinal fluid values were all normal The prot in level was 22 mg/100 ml and el ctrophoresis normal

To summari e this boy preterm and small for dates had a history well consistent with prenatal brain damage thought to be secondary to his mother s toxaemia during pregnancy When 6 years old he showed a clinical picture of dwarfism microcephaly with imbecillity severe motor retardation and congenital cataracts There was no evidence to support a diagnosis of a progressive encephalopathy He has had 6–12 hour episodes with vomiting and semicomatous states with or without simultaneous convulsions All episodes have occurred in the morning and 6 out of 12 followed a long car journey During the last 6 episodes a very low blood glucose (30 mg/100 ml) has been found

METHODS

The α ketoacids in the urine were determined quantitatively by means of th 2,4-dinitrophenylhydrazine reaction as d scribed by Natelson (24) The values are given in μmoles per g creatinine and α ketoisocaproic acid was used as a referen e

For the quantitative determinations of the amino acids two automatic amino acid analysers were used one being constructed at the laboratory according to the m thod d scribed by Spackman et al (30) and th other a commercial one (BIOCAL BC 200) Both analysers had been arranged so that analyses could be performed on 0 2 ml of serum or 1 0 ml of cerebrospinal fluid The urinary specimens were collect d in 24 hour samples Aliquots were taken and kept frozen at −20 C until analysed The blood specimens as well as the cerebrospinal fluid specimens were taken in the morning Th specimens were immediately centrifuged and the serum was deproteinized using 100 mg of solid sulphosalicylic acid for each sample of 2 ml of serum After thorough mixing the sample was centrifuged and the supernatant was kept frozen at −20 C until required for analysis

The urinary creatinine concentration was determined by the alkaline picrate method using the Technicon Auto Analyzer according to their method No N 11 b

Ins lin and growth hormone were determined by radioimmunoassays (32 33)

Blood glucose determinations were performed by an enzymatic method (16)

Cathecholamines were measured by a fluorometric method (11)

Enzymatic analyses of the branched-chain keto acid decarboxylase activity in the peripheral leuco yt s were performed by Profes or Joseph Dancis at the Department of Pediatrics New York Univ rsity Medical Center according to their method (3 4)

Loading tests

Insulin load 4 IU insulin per m of body area was given intravenously blood was analysed for glucose and growth hormone th urine was collected for the determination of cathecholamines

Bovril load 0 75 g per kg bodyweight of bovril was given orally Blood samples were analyzed for growth hormone (17)

Tolbutamide test 20 mg per kg bodyw ight of Tolbutamide was injected intravenously Blood samples were analysed for insuline

Leucine load 0 3 g L-leucine per kg body weight was given orally (13) Blood samples were analysed for blood glucose insulin and aminoacids

Isoleucine and valine loads 0 3 g isoleucine and valine respectively per kg body weight were given orally and blood samples were analy ed

Ketogenic diet was given as described by Col e & Ulstrom (2)

RESULTS

Table 1 shows the results obtained from quantitative amino acid analyses of specimens of serum urine and cerebrospinal fluid obtained from the patient on the 22nd of January 1969 when he was in a semisomnolent state The normal ranges as reported in the literature are



Fig 1 K S 4 5 years old (*a*) Sitting (*b*) creeping

weight of 10 3 kg He had now reached a motor developmental level of 7–8 months was able to sit unsteadily (Fig 1 *a*) and to creep slowly on all fours (Fig 1 *b*) He was unable to support himself in an upright position His speech was limited to 2–3 simple words (mainly Mammy and Daddy) He obviously functioned at an imbecile level with slow reactions to all stimuli General physical examination repeatedly showed normal findings Neurological examination revealed generalized dystonic changes with for instance scissoring in the upright position but he had no pareses and there was no hyperkinesia or ataxia All deep reflexes were brisk and the Babinski sign was negative In spite of bilateral discissions of his cataracts in 1965–1966 and correcting glasses his vision seemed to be poor However no changes in the ocular fundi were observed and the intraocular pressure was S/7 5/7 5 His hearing seemed to be normal During the period June 1967 – December 1968 this boy underwent five episodes with very peculiar semicomatose conditions lasting for 6–12 hours The evening before an episode he usually appeared unwell and did not want to eat Next morning he started to vomit continuously and gradually went into a lethargic state Two of the episodes were combined with recurrent tonic or clonic symmetric convulsions but the other three were free from convulsive characteristics Three of the episodes occurred in connection with long car journeys No particular smell from the urine was noticed during any of the attacks but his parents later stated (on direct questioning) that they had sometimes noted a faint peculiar smell of spice from the boy on other occasions The boy was thought to have some peculiar form of epilepsy and was put on phenobarbitone 30 mg per day

On the 21st of January 1969 the boy had his sixth and most severe episode At 7 a m he started to vomit violently half an hour later he went into a series of tonic convulsive states with stiffness of the arms and legs but with minor clonic movements of the eyes and jaw He became increasingly lethargic and was brought to the pediatric clinic There he was found to be unconscious but reacted to pain stimuli His eyes deviated to the left and his pupils reacted to light He had no more convulsions On arrival a screening acetic acid tube test on the urine was found to be positive + + + The boy woke up to some extent within a few hours but remained in a semisomnolent state for 36 hours However there were no difficulties in feeding him his general condition was good and he never became dehydrated

In the early morning of June 10th 1969 the boy had his seventh attack starting with repeated convulsions vomiting and lethargy His blood sugar on arrival at the hospital about one hour after the onset was 21 mg/100 ml Glucose (30 solution) was given immediately intravenously raising his blood sugar which was 61 mg/100 ml one hour later His convulsions stopped Five further episodes have occurred during the period 30 12 1969–20 7 1970 All episodes occurred in the mornings 3 of them following long motor car journeys The main symptoms were apathy or coma blood glucose varied between 18 and 25 mg/100 ml The most severe episode occurred on April 28th 1970 when the clinical picture was dominated by convulsions lasting for more than 30 min before he entered the hospital All episodes were treated with glucose (30 solution) intravenously and the patient improved almost immediately

EEG EEG recordings in free intervals were performed in 1965 1966 1968 1969 and 1970 and consistently revealed slight abnormalities with a somewhat slow basic activity for the age but no epileptogenic or dysrhythmic changes

ECG was repeatedly found to be normal during free intervals

X ray Except for the skull the whole skeleton showed signs of osteoporosis This was particularly marked in the spine and the pelvis and probably du to a marked muscular inactivity according to his cerebral palsy The ossification center development was within normal limits Intravenous urography showed normal findings

Laboratory findings in free intervals Routine blood values were all normal Blood pH 7 47-7 57 Pco 30 5-36 mmHg On different occasions serum phosphate was at a level of 3 2-3 4-4 4 mg/100 ml serum calcium at 4 7-4 8-4 9-5 0 mEq/l Serum electrolytes were all repeatedly normal Alkaline phosphatases were 4 4-6 2 B L units total bilirubin 0 9 mg/100 ml thymol 0 3 units GOT 30 units and GPT 10 units Fasting blood sugar in free intervals 57 90 113 mg/100 ml Plasma lipids Cholesterol 155 mg/100 ml phospholipid 199 mg/100 ml triglycerides 117 mg/100 ml

Intravenous glucose load (0 3 g glucose per kg bodyweight) showed a p rcentage disappearance per minute expressed as K_G value of 2 2 (corresponding to a half time of 32 min)

Urinary examination on repeated occasions excluded proteinuria and glycosuria The phosphat excretion was 370 mg/740 ml urine pho phate clearance was found to be 0 25-0 34 *ml/min*

Routine cerebrospinal fluid values were all normal The prot in level was 22 mg/100 ml and el ctrophoresis normal

To summarize this boy preterm and small for dates had a history well consistent with prenatal brain damage thought to be secondary to his mother s toxaemia during pregnancy When 6 years old he showed a clinical picture of dwarfism microcephaly with imbecillity severe motor retardation and congenital cataracts There was no evidence to support a diagnosis of a progressive encephalopathy He has had 6-12 hour episodes with vomiting and semicomatous states with or without simultaneous convulsions All episodes have occurred in the morning and 6 out of 12 followed a long car journey During the last 6 episodes a very low blood glucose (30 mg/100 ml) has been found

METHODS

The α ketoacids in the urine were determined quantitatively by means of the 2,4-dinitrophenylhydrazine reaction as d scribed by Nat lson (24) The values are given in µmoles per g creatinine and α k toisocaproic acid was used as a reference

For the quantitative determinations of the amino acids two automatic amino acid analysers were used one being constructed at the laboratory according to the method d scribed by Spackman et al (30) and the other a commercial one (BIOCAL BC 200) Both analysers had been arranged so that analyses could be performed on 0 2 ml of serum or 1 0 ml of cerebrospinal fluid The urinary specimens were collect d in 24-hour samples Aliquots were taken and kept frozen at −20°C until analysed The blood specimens as well as the cerebrospinal fluid specimens were taken in the morning Th specimens were immediately centrifuged and the serum was deproteinized using 100 mg of solid sulphosalicylic acid for each sample of 2 ml of serum After thorough mixing the sample was centrifuged and the supernatant was kept frozen at −20 C until required for analysis

The urinary creatinine concentration was determined by the alkaline picrate method using the Technicon Auto Analyzer according to their method No N 11 b

Insulin and growth hormone were determined by radioimmunoassays (32 33)

Blood glucose determinations were performed by an enzymatic method (16)

Cath cholamines were measured by a fluorometric method (11)

Enzymatic analyses of the branched-chain keto acid decarboxylase activity in the peripheral leuco yt s were performed by Professor Joseph Dancis at the Department of Pediatrics, New York Univ rsity Medical Center according to their method (3 4)

Loading t sts

Insulin load 4 I U insulin per m of body area was given intravenously blood was analysed for gluco e and growth hormone th urine was collected for the determination of cathecholamines

Bovril load 0 75 g per kg bodyweight of bovril was given orally Blood samples were analyzed for growth hormone (17)

Tolbutamide test 20 mg per kg bodyw ight of Tolbutamide was injected intravenously Blood samples were analysed for insuline

Leucine load 0 3 g L-leucine per kg body weight was given orally (13) Blood samples were analysed for blood glucose insulin and aminoacids

Isoleucine and Valine loads 0 3 g isoleucine and valine r spectively per kg body weight were given orally and blood samples wer analy ed

Ketogenic diet was given as described by Col e & Ulstrom (7)

RESULTS

Table I shows the results obtained from quantitative amino acid analyses of specimens of serum urine and cerebrospinal fluid obtained from the patient on the 22nd of January 1969 when he was in a semisomnolent state The normal ranges as reported in the literature are

Table 1 *Serum, urinary and cerebrospinal fluid amino acids in specimens obtained on 22nd of January 1969*

Amino acid	Serum (mg/100 ml) 22 1 1969	Serum Normal range (ref 26)	Urine (mg per 24 hrs) 22 1 1969	Urine Normal range (ref 31)	Cerebrospinal fluid (μM/100 ml) 22 1 1969	Cerebrospinal fluid Normal range (ref 8)
Taurine	2 28	0 71–1 44	54 7	86–294	0 7	0 22–0 94
Aspartic acid	0 19	0 05–0 27	3 5	<10		0 03–0 22
Threonine	2 26	0 50–1 13	10 6	15–53		0 77–4 91
Serine	1 84	0 83–1 18	Not separated	27–73	3 13	1 65–12 11
Asparagine + Glutamine	14 87	8 3–68 0	8 7	34–92		19 4–86 5
Proline	3 53	0 78–1 70	—	<10	Trace	0–0 58
Glutamic acid	0 33	0 34–3 68	10 3	8–40	9 22	0 24–2 46
Citrulline	0 23	0 21–0 52	Trace	—	—	0 11–0 45
Glycine	1 46	0 88–1 67	35 5	68–199	0 8	0 40–1 07
Alanine	1 38	1 22–2 72	6 4	21–71	1 52	1 17–4 46
Valine	6 69	1 50–3 31	0 6	4– 6	2 52	0 74–2 82
Cystine	0 84	0 54–0 91	13 1	10–21	Trace	Trace
Methionine [a]	0 59	0 16–0 24	Trace	<5–10	0 19	0 05–0 75
Isoleucine	3 17	0 37–1 1	1 3	14–28	1 11	0 30–0 86
Leucine	5 07	0 73–2 32	1 9	9–26	2 37	0 62–2 22
Tyrosine	2 99	0 50–1 28	11 1	15–49	1 13	0 30–2 43
Phenylalanine	1 50	0 43–1 01	1 9	9–31	1 10	0 16–2 74
Ornithine	0 79	0 36–1 14	7 5	—	0 32	0 37–1 02
Lysine	2 10	1 04–2 20	102 1	7–48	0 96	0 61–3 34
Histidine	0 98	0 37–1 32	88 1	113–320	0 32	0 62–2 38
Arginine	0 97	0 40–1 50	3 6		Trace	0 80–2 91
1 methylhistidine			13 5	47–384		
3 methylhistidine			12 6	50		

[a] Methionine + alloisoleucine

also given for comparison It is seen in the table that there was an especially marked increase of the serum levels of valine isoleucine and leucine but that the threonine serine, proline phenylalanine and tyrosine levels in the serum were also elevated The slight increase in the serum methionine concentration (0 59 mg/100 ml) might be due to an increase in the alloisoleucine level, since this amino acid cannot be separated from methionine by the method used No pathological amount of any amino acid was found in the urine In the cerebrospinal fluid the isoleucine and leucine levels were pathologically increased whilst the valine concentration lay in the upper part of the normal range

Since small samples had been kept from the specimens of serum urine and cerebrospinal fluid obtained on earlier occasions it was possible to perform a longitudinal study of some of the biochemical alterations in these fluids

The findings presented in Table 1 suggested that the boy might have some type of branched chain aminoacidemia It was therefore considered of interest to study the keto acid excretion Urinalyses revealed an increased urinary excretion of α ketoacids during the acute stages of the episode in January 1969 (1 550 μM/g creatinine) and in June 1969 (1 530 and 2 107 μM/g creatinine) There was no distinct α ketoacidemia (80–300 μM/g creatinine) found in any of the urinary specimens obtained during free intervals

Growth hormone function was shown to be normal with an increase of growth hormone in serum during the bovril test from 3 7 ng/ml to 16 0 ng/ml after 90 min During insulin loading no significant increase was noted in spite of adequate hypoglycaemia (blood glucose <40 mg/100 ml) however the starting levels were rather high On two occasions growth hormone levels exceeding 20 ng/ml

were observed during spontaneous hypoglycaemia

Insulin levels did not increase significantly during leucine loads on Tolbutamide an increase from 2 5 μE/ml to 67 μE/ml was noted which has been judged a normal response

Epinephrine excretion was 3 3 μg/4 hours following the insulin load compared with 0 45 μg/hour in normal condition The increase of norepinephrine during insulin load was of about the same range

Glucagon (0 5 mg i v and 1 mg i m) was given on two different occasions when the patient was in a hypoglycaemic state after starvation However no increase in blood glucose levels was noted within 20 min

On ketotic diet ketonuria was noted within 9 hours 18 hours after the first meal the blood glucose was 17 mg/100 ml and the patient was in a semicomatous state He recovered rapidly on the administration of glucose intravenously

The findings during ketogenic diet are summarized in Fig 2 and showed an increase in the plasma concentration of the branched chain amino acids as well as an increased urinary excretion of α keto acids As seen in Table 2 these findings were in complete agreement with those obtained during spontaneous hypoglycaemic episodes whilst the plasma aminogram as well as the urinary excretion of α keto acids were within normal range during free intervals

Since a disturbance in the branched-chain amino acids was suspected a provocation test with a normal caloric – high protein diet was given (70 kcal and 4 48 g protein per kg body weight) during 3 days This period was followed by a high caloric diet with the same protein content (140 kcal and 4 9 g protein per kg bodyweight) during 4 days During the first period an increase of the branched chain amino acids were observed (leucine 5 3 mg/100 ml isoleucine 3 2 mg/100 ml valin 7 9 mg/100 ml) During the second period however the value of these amino acids were within normal ranges

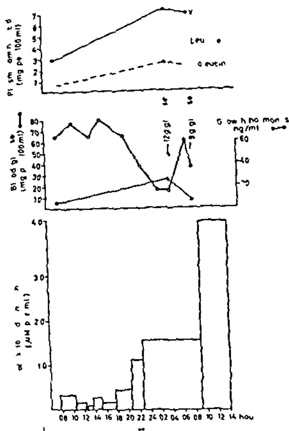


Fig 2 Biochemical findings during provocation of hypoglycaemia with ketotic diet

The results obtained during loading tests with leucine isoleucine and valine are shown in Fig 3 It is seen that there was no effect on the blood glucose level nor was there any pathological response with respect to the plasma levels of leucine isoleucine and valine

Enzymatic analyses of the branched-chain keto acid decarboxylase activity in the peripheral leucocytes showed a somewhat reduced activity It was however within the range which is found in asymptomatic and presumed normal individuals

DISCUSSION

Clinical findings

Our patient's habitual clinical condition between his attacks represents the characteristic picture of a "small for date" child with pre

Table 1 *Serum urinary and cerebrospinal fluid amino acids in specimens obtained on 22nd of January 1969*

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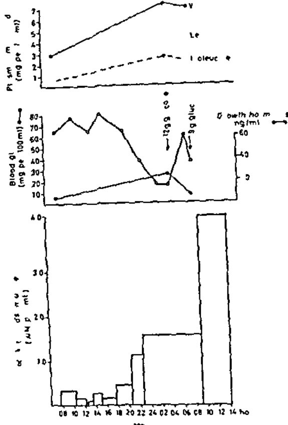


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DISCUSSION

Clinical findings

Our patient s habitual clinical condition between his attacks represents the characteristic picture of a "small for date child with pre

Table 2 *Plasma amino acids and α keto acids in urine during episodes of hypoglycaemia and during free interval*

Amino acid	Free intervals Mean	*s*	*n*	Spontaneous hypoglycaemia Mean	*s*	*n*	Hypoglycaemia provoked by ketotic diet	Normal range (ref 26)
Plasma amino acids (mg per 100 ml)								
Taurine	1 15	0 34	11	2 03	0 97	6	1 39	0 71–1 44
Aspartic acid	0 40	0 21	8	0 20	0 01	2		0 05–0 27
Threonine	1 06	0 37	8	1 17	0 94	3	0 33	0 50–1 13
Serine	1 54	0 38	8	0 92	0 50	6	0 60	0 83–1 18
Glutamine	6 51	4 20	8	8 23	3 67	6	7 15	8 3–68 0
Proline	2 00	1 06	9	1 23	1 30	5	1 01	0 70–1 70
Glutamic acid	3 03	2 10	10	1 55	1 19	5	0.22	0 34–3 60
Citrulline	0 27	0 18	10	0 16	0 16	5	Trace	0 21–0 52
Glycine	1 68	0 35	11	0 87	0 39	6	0 64	0 88–1 17
Alanine	2 32	0 57	11	1 14	0 38	6	1 06	1 22–2 72
Valine	2 38	0 87	10	5 48	1 93	6	7 34	1 50–3 31
Cystine	1 04	0 52	7	0 96	0 20	5	1 55	0 54–0 91
Methionine	0 32	0 29	10	0 18	0 20	6	0 13	0 16–0 24
Isoleucine	0 79	0 30	11	2 58	0 96	6	2 61	0 37–1 10
Leucine	1 46	0 69	11	4 68	1 50	6	4 99	0 73–1 32
Tyrosine	0 99	0 62	11	0 89	1 04	6	0 63	0 50–0 28
Phenylalanine	0 85	0 31	11	0 70	0 43	6	0 58	0 43–1 01
Ornithine	0 91	0 36	6	0 66	0 17	6	1 04	0 36–1 14
Lysine	2 29	1 06	6	1 64	0 29	6	1 49	1 04–2 20
Histidine	0 76	0 30	6	1 30	0 26	6	0 98	0 37–1 32
Arginine	0 97	0 39	6	0 84	0 25	6	0 53	0 40–1 50
α keto acids in urine (M per g creatinine)	145	76	10	1795	971	5	1703	

natal brain damage and a tendency to spontaneously occurring hypoglycaemic episodes However his seventh attack was obviously associated with hypoglycaemia and so were all the following episodes The fact that many episodes have been provoked by car journeys is of special interest The feeding during these journeys has been short acting carbohydrates and fat mainly sweets lemonades and chocolate On the evening after the journey the patient has refused to eat and on the following morning a hypoglycaemic state appeared No

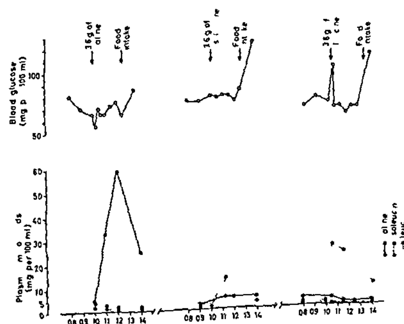


Fig 3 Alterations in blood glucose and plasma amino acid concentration of leucine iso eucine and valine during loading tests with leucine isoleucine and valine

endocrine disturbances affecting growth hormones insulin epinephrine and norepinephrine have been found Glucagon was not able to increase blood glucose levels in hypoglycaemic states indicating that the depot of glycogen were emptied Leucine did not increase insulin levels nor did hypoglycaemia appear The only positive provocative test was the ketotic diet During this test a marked hypoglycaemia appeared within 18 hours and the clinical findings were in good agreement with his spontaneous episodes The onset of the symptoms at the age of 3 years is typical for ketotic hypoglycaemia whilst types of chronic hypoglycaemia normally have an onset before the age of 2 years From the dietetic point of view the diets before the spontaneous episodes occurring in relation to car journeys are rather similar to the ketotic diet which provoked hypoglycaemia

The clinical picture during the acute attacks with vomiting initial convulsions and a long lasting lethargic state together with biochemical evidence of a transient inability to metabolize the branched-chain keto acids suggested a diagnosis of transient MSUD It is evident however that the serum concentrations of the branched chain amino acids valine isoleucine and leucine as well as the urinary excretion of α keto acids never reached such a high level in this case as has been reported in typical cases of branched chain ketonuria reported by other authors (6 7 12 19 20 28 29) Even in reported cases of transient forms of MSUD (4 18 22) where quantitative data have been given the maximum values have been higher than those found in our patient This indicates that our patient does not represent a case of transient MSUD On the other hand it is quite obvious that the plasma levels of the branched-chain amino acids found are abnormal

It is of interest that the other abnormalities of the serum amino acid pattern found in our patient such as elevations of serum threonine serine proline tyrosine and phenylalanine completely agree with the findings of Snyderman and her collaborators in their studies of cases of MSUD (28 29) However in some other aspects our results differ Snyderman in 11 cases of MSUD found a much greater degree of elevation of leucine in the serum than of the other branched-chain amino acids (28) The same finding has been reported by other authors (7 12 25) However in our patient there is no pronounced difference between the branched-chain amino acids with respect to their elevation in relation to the average plasma values

Further evidence that this is not a case of MSUD constitutes the findings that no disturbance in the plasma aminogram was seen when a high caloric – high protein diet was given This suggests that the increase obtained during normal caloric – high protein diet was not provoked by the high protein intake but by the ketogenic effect of this diet

Hypoglycaemia in association with MSUD has been described by several authors (9 19 20 27) and several investigators have speculated whether or not this mechanism resembles that of the leucine induced hypoglycaemia originally described by Cochrane (1)

Our observations in this patient and a critical analysis of published cases of hypoglycaemia in MSUD give rise to the hypothesis that some of these are more probably cases of ketotic hypoglycaemia rather than hypoglycaemia related to MSUD

This patient differs from typical cases of ketotic hypoglycaemia as well as from cases of the variant form of MSUD by his severe mental retardation a syndrome of cerebral palsy and cataracts The prenatal history his neonatal signs the course of his psychomotor development and the neurological signs are well in agreement with a prenatal brain damage due to toxaemia in his mother

SUMMARY

A 6-year-old boy with a clinical picture of dwarfism microcephaly with imbecillity severe motor retardation and congenital catar

acts is described The patient was born preterm and small for date and had a clinical history consistent with prenatal brain damage and was in a bad condition with convulsions and ab normal neonatal neurology

The patient had periods of semicomatose states with or without simultaneous convul sions During these episodes it was possible to show hypoglycaemia as well as increased uri nary excretion of α ketoacids and an increase of the plasma levels of leucine isoleucine and valine

A provocative test using a ketogenic diet brought the patient into a clinical condition similar to his spontaneous episodes Biochem ically not only the typical findings of hypo glycaemia and ketonuria but also of an in creased plasma level of branched chain amino acids as well as of an increased urinary excre tion of α ketoacids was observed during the provocation

ACKNOWLEDGEMENTS

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REVIEW ARTICLE

A STUDY OF ADOPTED CHILDREN, THEIR BACKGROUND ENVIRONMENT AND ADJUSTMENT

MICHAEL BOHMAN

From the Child Psychiatric Department of Karolinska Institutet at St Görans Hospital (Head Sven Ahnsjö) Stockholm and the Child Welfare Committee of Stockholm Sweden

This paper summarizes the results of a study of adopted children and their families that was conducted by me during the years 1965–1968 and which has been published elsewhere (2)

Studies on the process of adoption have been stimulated mainly from two quarters Genetic research and the child welfare authorities of the community For genetic research the adoptive situation presents a ready made experiment for testing hypotheses concerning the relative importance of heredity and environment Such studies were done in the twenties and the thirties by Freeman and co workers (8), Burks (6), Leahy (17) and more recently by Heston & Denney (10) Kety et al (15) Rosenthal et al (21) and Schulsinger (25) On the other hand the goal of social research into adoption is to determine which factors in the child, the adoptive parents and society influence the adoptive process and to what extent this process can be forecast prior to placement

It is generally accepted that adoption affords society an instrument for social therapy and the responsibility of placing children without parents is considered to call for a high level of knowledge and decision making Despite this few representative studies have been made to throw light on the various aspects of adoption An extensive survey of adoption research by Pringle concerning the years 1948–1965 (20) mentions only three major studies involving non clinical populations of adopted children all in the United States Most studies on adoption (that is not specifically concerned with genetic questions) are on small clinical populations whose selection is not reported The majority of studies have been exploratory and designed to tackle some particular aspect of adoption There are also a good many contradictions between their results though there seems to be a certain amount of agreement on the following points

1 Placement of an adoptive child should be undertaken as early as possible though there is still disagreement as to whether this means during the third and fourth weeks of life rather than during the first or even the second year (20)

2 Environmental factors appear to be more important than genetic factors for the development of the child s general character whereas the greater part of the variation in intelligence among groups of adopted children is attributable to genetic factors (17)

3 The personal characteristics of the adoptive parents and their mutual relations have the greatest bearing on the outcome of an

adoption while their ages income socio-economic group and education appear to be of secondary importance (28)

4 It is agreed that the child should be informed about the situation but opinions differ as to the age at which the child should first learn about its adoptive status (5 16 18 23 24)

5 Adopted children appear to be over represented in various clinical populations and this has been interpreted as indicating that they are more vulnerable to stress than children in general (4 9 11 12 14 19 22 23 26 27)

6 Tests during infancy are seldom able to predict the child s subsequent development (1 29)

Little research has been done on the importance of genetic pre natal and peri natal factors for the child s development and not much is known about how society s attitudes to adoption affect the adoptive family

The follow up study

The purpose of the study was to investigate questions connected with the placement of adopted children and to codify past experience as a foundation for future adoptions and was conducted on 168 adopted children (93 boys and 75 girls) representing all children born during 2 years in the mid fifties and placed in adoptive homes by the Stockholm Adoption Agency The children were 10–11 years old at the time of the follow up Most of the families (124) were living in Stockholm and a smaller group (40) were living in other parts of Sweden the remaining 4 families were living abroad

The investigated group of children is the result of many selective factors For various reasons the agency did not or could not place all children whose mothers had applied for an adoption Of all 624 children born during the 2 years and registered for adoption about a third returned to their mothers who had changed their minds about adoption and lived there at the time of the follow up 10 years later Another third had because of certain selective factors been placed in foster homes where some had been legally adopted This leaves only 27% of the primary series placed in adoptive homes selected by the agency Table 1 gives an account of the various placement groups at the time of the follow up All children were born after an undesired and socially complicated pregnancy and measures of decisive importance for their future were as a rule taken before they were 1 year old This similarity made it interesting to compare the development and the adjustment of children *in different placement categories* Consequently the study was planned so that these other groups were included in certain parts of the follow up The results of these comparative studies will be published later (3) but some of the results are given also in this paper

Information was obtained from previous investigations and records as well as from official registers but chiefly through interviews with adoptive parents and teachers The interviews with parents covered 122 of the 124 families living in Stockholm (2 families refused to participate) The interviews with teachers covered all but 5 of the children in the study Information was also obtained about the children s school marks and their general health according to the school health cards A control group was obtained in the form of classmates of the same sex and certain comparisons were also made with all classmates of the same sex The information obtained from the adoptive parents was compared with corresponding data from a representative study of Stockholm boys and their parents by Jonsson & Kalvesten (13)

Pre and perinatal conditions

The mean and range of the adopted boys weight at birth displayed good agreement with a normal Swedish population On the other hand the mean birth weight of the adopted girls were significantly lower than expected Only 5 children were born prematurely (3%) This unexpectedly low figure was the result

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Table 2 *Adopted children s adjustment to school compared with controls (percentages)*

Adjustment	Boys: Adopted ($N=90$)	Boys: Controls ($N=51$)*	Girls: Adopted ($N=73$)	Girls: Controls ($N=44$)*
1 No symptoms	29	56	62	75
2 Slight symptoms	14	14	15	9
3 Moderate symptoms	35	18	12	11
4 Problem child	20	12	11	5
5 Institutional case	2	0	0	0
Total	100 A	100 B	100 C	100 D

Diff A B χ = 11 71 (3 df) $p<0\ 01$ Items 4 and 5 combined
Diff C D χ = 2 84 (3 df) Items 4 and 5 combined
Diff A–C χ = 20 95 (3 df) $p<0\ 001$ Items 4 and 5 combined

died while the study was in progress One adopted child in five appears to have had at least one parent whose physical health was impaired More than one child in four had a parent with a history of mental disturbance that had led to medical consultation for nerves The adoptive mothers reported physical or mental trouble more frequently than the adoptive fathers According to their own statements however the physical and mental status of the adoptive parents was better than that of the parents in the comparative group but even so the number with an impaired physical or mental condition does seem large considering that the adoptive parents as a group were assumed to represent a positive selection in respect of health

In 19 of the adoptive families the marriage had been dissolved by divorce and in 4 by the death of one of the parents Two more parents died while the study was in progress Thus about one adopted child in six had lost one of its adoptive parents The proportion of incomplete homes due to divorce or death was approximately the same among ordinary Stockholm families in the comparative group The interviews showed in retrospect that in most of the divorce cases conflicts and disharmony—expressed for instance in problems of sexual adjustment—had already existed when the child was placed In only a few cases however had this state of affairs been noted in the investigation that preceded placement

The adoptive parents attitudes to various aspects of their life (marriage work financial situation relatives religion leisure etc) were measured from the interviews on attitude scales and compared with corresponding results for the comparative group of Stockholm families Most of the adoptive parents had very positive attitudes in the case of prevailing marriages and good agreement was noted between the partners statements Similar results were obtained for sexual adjustment In other respects too the majority of adoptive parents indicated an affirmative attitude to life and seldom expressed dissatisfaction with their situation They differed significantly in most categories from the parents in the comparative group Social relations and social life were the only aspects that appeared to cause any appreciable dissatisfaction

Concerning the adoptive parents own upbringing 20–50% considered that this had been strict as against 70–75% in the comparative group Thus most of the adoptive parents appear to have had a relatively mild upbringing and as a consequence of this only a few of them had a negative attitude to their own upbringing

The adoptive parents attitudes to upbringing were measured with a questionnaire com

of a positive selection, since most prematurely born children in the primary series were not placed in adoptive homes by the agency Toxemia including cases with no more than elevated blood pressure was present in about 20% of the births and appears to have been over represented in the adoptive series, compared with normal births

Institutional care

Prior to placement, all but 6 of the children were cared for at an infants home for varying lengths of time Rather more than half of the children had been placed in an adoptive home before they were 6 months old 10% were placed during the last quarter of their first year and only one was a year old at placement

The biological parents

The majority of the women who had their child adopted were young unmarried or living alone Only about one in four of the mothers had a home of her own About half had previously given birth to one or several children Financial circumstances and lack of housing were cited by most of the mothers as reasons for having the child adopted in addition to the purely personal reason that was probably decisive in most cases namely lack of support from the child's father About one fifth considered that a major reason for leaving the child was society s moral condemnation of illegitimate birth

Information was obtained on 150 fathers paternity not having been established in the other 18 cases Most of the fathers were Swedish citizens (85%) and the others belonged to the European ethnic group Their mean age 28 55 years was somewhat lower than in a representative group of fathers Some idea of the biological fathers social conduct was obtained by studying the registers concerning abuse of alcohol and crime The proportion of biological fathers registered for abuse of alcohol 27%, was somewhat higher than in a representative group of fathers The proportion of registered criminality was 27% as against 11% in the comparative group There was also a tendency to greater recidivism compared with normal fathers registered for crime and this may have to do with the higher proportion of the present fathers who appeared in both registers, for abuse of alcohol and for crime

Table 1 *Whereabouts of the 624 children in the primary series at the time of the follow up Children 10–11 years*

Whereabouts	No children
Adopted through the Adoption Agency in Stockholm (Agency Adoptions)	168
Adopted without Agency assistance	1?6
Foster home	77
With biological mother	228
At an institution	3
Other placement	6
Dead	14
Unknown	2
Total	624

The adoptive parents

When the children were placed in their care the adoptive parents were about 35 years old on the average and 6–7 years older than a representative group of Stockholm parents Concerning the occupation of the family provider (usually the adoptive father) there were 34% with professional occupations 37% with intermediate and 29% with skilled unskilled occupations (according to the official British classification (7) modified somewhat for circumstances in Sweden) The two most qualified occupational groups were thus heavily over represented among the adoptive families The average educational level of the adoptive parents was higher than that of the fathers in the comparative group and considerably more of them had also advanced socially

The health of the adoptive parents was assessed from their own or the other parent s statements Two adoptive fathers and 2 adoptive mothers had died of some disease while a further adoptive father and an adoptive mother

Behaviour as perceived by the teachers

The occurrence of certain readily-defined behavioural characteristics among the adopted children was studied without the teacher being aware of the subject s identity among classmate populations of the same sex As perceived by the teachers the adopted boys were significantly more lively than their classmates were in conflict with their peers more frequently and had a lower status As regards intelligence on the other hand the adopted boys displayed the same distribution as their classmates The adopted girls deviated considerably less than the boys from the behaviour of their classmates They had conflicts with peers somewhat more often than their classmates of the same sex and were also perceived by the teachers as more industrious and ambitious than other girls

Adjustment in school

Among the adopted boys 22% were judged to be maladjusted in the school situation compared with 12 of the controls while a further 35% had moderate symptoms compared with 18% of the controls The difference between the groups was significant

Problems and difficulties of adjustment also occurred more frequently among the adopted girls compared with their class controls but the difference was less pronounced and not significant It was considered that 11% of the adopted girls were problem cases compared with 5% of the controls The adopted boys were significantly less well adjusted than the adopted girls Compared with the controls a significantly larger proportion of the boys as well as the girls displayed multiple symptoms The results are summarized in Table 2

The teachers and the parents differed considerably in their perception of the children s general adjustment as well as in their observation of individual symptoms Thus many more symptoms were registered during the interviews with teachers than during those with parents The adoptive parents were considerably less prone to mention the child s problems even if these existed in the school situation The differences between the interviews with teachers and with parents were particularly marked in the case of families with internal problems

Asocial symptoms (truancy vagrancy lying stealing and pilfering destructiveness) were reported relatively seldom by the adoptive parents or teachers and do not appear to have been more common among the children than in a normal group of children The boys who were problem children" were chiefly characterized by a cluster of the following symptoms psychomotor hyper activity inability to concentrate disturbed relations with peers defiance and aggressiveness

GENERAL ANALYSIS

Before ascertaining the data outlined above numerous hypotheses had been set up in accordance with certain theories that attempt to explain variations in the adjustment and behaviour of children as being dependent on different background variables Briefly test of these hypotheses produced the following results

1 Conditions registered during pregnancy and birth displayed no significant relationship with the children s adjustment or school marks Complications during pregnancy did however show a significant relationship with difficulties in reading and writing The children who were classified as maladjusted also had wider variations in weight at birth than the other children but the differences were not significant

2 The time spent at an infants home showed no significant relationship with the children s adjustment A regression analysis revealed a weak linear relationship between the duration of institutional care and the number of symptoms registered Difficulties in reading and writing were more common among boys who had spent more than 6 months at an infants home than among those whose stay had been shorter ($p < 0.05$ one tailed test)

prising seventeen statements that had also been used in the study of the comparative group On average, the adoptive parents attitudes to upbringing were milder' than those of the comparative group The questions on which the groups differed most concerned corporal punishment and sexual behaviour in the child the adoptive parents expressing more liberal views The two groups of parents had fairly similar attitudes on the other hand concerning obedience and discipline

Type of upbringing

The author made a subjective evaluation of the type of upbringing practised by the parents (level of demands) The demands of the adoptive fathers were relatively high in the case of boys but not girls whereas the adoptive mothers placed roughly the same relatively high demands on boys as on girls A significant relationship was also found between the level of demands' of the adoptive fathers and their own upbringing as a child so that fathers who had received a mild upbringing expressed more adequate demands than those who had received a strict upbringing

Disclosure of adoption

Of the 164 couples who were asked orally or in writing whether they had disclosed the fact of adoption to the child 68% stated that they had done so before the child was 5 years old 13% between 5 and 7 years and 4% after 7 years Of the remainder 7% stated that they had not wished or could not bring themselves to inform the child that it had been adopted and 7% refused to answer the question Most of the children in the latter group were probably completely or partially unaware that they were adopted

Infertility

Childlessness was due to infertility in the adoptive mother alone in 45% of the cases and in the adoptive father alone in 15% Both partners were reportedly infertile in 9% The cause of childlessness was not known in spite of examinations in 20% of the cases while only an incomplete or no examination had been undertaken in 6% In the remaining 5% the childlessness was said to be voluntary owing to the use of contraceptives for medical or genetic reasons Assuming that male infertility is as common as female it seems that the considerably higher incidence of infertility among the adoptive mothers may reflect different attitudes to infertility and adoption among men compared with women In marriages where the husband is infertile, it seems *that the problem of childlessness is resolved* by an adoption less frequently than when the woman is infertile In the decade between the placements and the present study 17 live births had taken place in 13 of the families (8%) Five of these children were born to the couples who practised contraception voluntarily for medical or genetic reasons

The adopted children

Nearly all the adopted children were in good health Two of them however had severely impaired hearing probably congenital but not diagnosed until after the child had been placed with its adoptive parents

School performance

About 6% of the adopted children attended special classes (for slow learners assistance in reading and writing etc) which is about the usual frequency among school children in Sweden None of the children was mentally retarded The mean marks of the adopted children in Swedish and mathematics were compared with the means for class populations of the same sex No difference was found in the case of Swedish but in mathematics the adopted children had a lower mean mark The difference was only significant however for the children attending school in the City of Stockholm No differences were observed between the mean marks of the adopted boys and the adopted girls

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3 A weak, positive correlation was found between the mothers' education and the girls marks in Swedish ($p < 0.05$ one tailed test) Psychological or social insufficiency in the biological mothers was not significantly associated with the adjustment among the children

4 *The occurrence of the biological fathers* in the registers of crime or abuse of alcohol was not related to the children s adjustment It may be concluded that the children's social heritage so far had been neutralized in their new adoptive homes The results do not confirm or exclude that genetic factors may be of etiological importance for the development of nervous symptoms in children That genetic factors may be of some importance was shown in the study of those children from the primary series who were placed in foster homes or had returned to their biological mothers In these groups I found a significant correlation between the adjustment of girls and the criminality of their biological fathers On the other hand there was no such correlation between the boys and their fathers

5 The education and occupational group of the adoptive parents were not correlated as expected with the children s school performance if anything the results pointed in the opposite direction The children who had *been placed with the most qualified adoptive* parents had lower mean marks than other children

6 Neither the age nor the socio economic circumstances of the adoptive parents correlated with the adjustment of the adopted children

7 The adoptive mothers attitude to marriage co varied significantly with the adjustment among the children but this was not true of the attitude of the adoptive fathers The attitudes of both parents to social contacts (friends acquaintances) also co varied significantly with the children s adjustment The mental status of the adoptive mothers showed a weak correlation with the boys adjustment

8 The adoptive parents own upbringing as children did not display the expected relationship with the children s adjustment In the case of the adoptive fathers the result pointed if anything in the opposite direction—the fathers who had had a mild upbringing had less well adjusted children than those who had been brought up strictly The attitudes of the adop*tive parents to upbringing were not signifi*cantly associated to the adjustment of the children A significant association was observed on the other hand, between the adoptive parents type of upbringing (level of demands) as estimated at the interviews and the adjustment of the boys, but not with the adjustment of the girls

9 Maladjustment was significantly more common among boys who were the only child than among the other boys but this was not true of the adopted girls

In conclusion it may be noted that the adjustment of the children appeared to be relatively independent of the background variables investigated Neither the environmental factors studied nor those connected with the biological background displayed very clear correlations with the children s adjustment or school performance There is reason to suppose that the over representation of behavioural disturbances observed among the boys in particular may be connected with the adoptive situation itself *and the disturbances that this involves* in the relationship between parents and child It is conceivable that these disturbed relationships were connected with an uncertainty on the part of adoptive parents in their attitude to adoption expressed by many of them in a *desire to adopt a girl rather than a boy*

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Fig 1 Face Note the confluent hemorrhagic and necrotic lesions of the varicella.

Blood serum proteins were 4 1 g/100 ml and albumin 2 6 g/100 ml

The electrophoresis showed albumin 65 1 and globulins alfa 5% alfa 10 6% beta 16 9% and gamma 3 3% The absolute value of the gamma globulin was 135 3 mg/100 ml Immunoglobulins of the *serum* (before application of gamma globulin and reconvalescence serum 11th day of illness) are presented in Table 1 (evaluated according to double immunodiffusion method of Mancini) The IgM and IgG are a little low as compared with healthy children and much lower than in another child of the same age in convalescence after varicella Apparently the immunoglobulins in our patient did not rise as expected in the course of the varicella The urine was normal on admission After a week general edema appeared and the child was *oliguri* Only 20 cc of urine could be collected during 2 days, and showed protein + + + + and the sediment showed full field of erythrocytes and leukocytes 1-2 granulated cylinders were found in a ×50 high power field The findings remained the same on the day of death (23 9 69) when only 15 cc of urine was collected The blood urea on 22 9 69 was 21 mg/100 ml with Na = 139 mEq/l K = 3 7 mEq/l Cl = 98 mEq/l On 23 9 69 the urea rose to 60 mg/100 ml The electrolytes on this day were Na = 142 mEq/l K = 3 75 mEq/l and Cl = 102 mEq/l. The blood pressure on his last 2 days of life remained in the range of 80-85 mmHg systolic and 55 diastolic pressure

The child was treated with antibiotics such as Cloxallin and Ampicillin, 5 ml of gamma globulins and because of his deterioration, he received after a week 9.5 ml of convalescent serum of his sister who had varicella about a month before The child died on 23.9 69

Autopsy

The external examination of the body revealed the dense papulo-vesicular eruptions over the entire body including face and extremities

The lobes of both lungs showed the same changes i e dark red firm areas alternating with greyish yellowish dense zones

No pathological changes in other organs were found except for the thymus which was very small and could be determined only by palpation being situated upon the pericardium

Microscopical examination The skin changes were suggestive of varicella The lesions showed different evolutionary stages ranging from very recent to old There were cells with ballooning degeneration, necrosis and vacuole formation in the epidermis In some cells situated at the periphery of the vacuoles homogenous acidophilic intranuclear inclusion bodies were found surrounded by a clear halo and the nuclear membrane seemed thickened (Fig 2) In the dermis there was a dense leukocytic infiltration mostly present around the blood vessels

Lungs Multiple sometimes confluent, necrotic zones without inflammatory reaction were present. On the periphery of the necrotic zones in some enlarged alveolar cells eosinophilic intranuclear inclusion bodies were found In the preserved zones there was some mononuclear infiltrate in the alveolar septa and a large number of desquamated alveolar cells in the alveolar lumina could be seen These cells contained one or more nuclei but they did not show inclusion bodies

Table 1 *Immunoglobulins of serum*

Case	Age (months)	IgG (mg/ 100 ml)	IgA (mg/ 100 ml)	IgM (mg/ 100 ml)
Our case (14 days after onset of varicella eruption)	7	340	38	21
Other child with varicella (14 days after onset)	7 5	1 250	212	110
Normal values[a] of healthy children	7	425±75	30±16	40±10

[a] Fulginiti V A et al *J Pediat 68* 723 1966

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CASE REPORT

VARICELLA AS THE CAUSE OF DEATH IN AN INFANT AFFECTED BY LYMPHOPENIC THYMIC DYSPLASIA WITH DYSGAMMA-GLOBULINEMIA

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Thymic dysplasia combined with lymphocytopenia usually referred to as Swiss type agammaglobulinemia was first described in 1950 by Glanzmann & Riniker (7), and subsequently by other authors (6, 9 10 15, 19, 21) Fireman et al (4) described in 1966 a similar condition where only dysglobulinemia was found and called the disease Thymic alymphoplasia with dysgammaglobulinemia ' Children affected by these two conditions are very susceptible to recurrent infections of bacterial and viral origin

CASE REPORT

The subject is a 7 month old boy of 3 250 g birth weight The father was a Jew from Argentina and the mother from England They were healthy and there was no consanguinity They had 3 other children (one girl and two boys) one of whom suffered from asthma There were no special diseases and no sensitivity to infections in either parental family Hematological examinations of the parents and the siblings were normal (number of leukocytes lymphocytes and electro phoresis and immunoelectrophoresis of the serum)

Pregnancy and delivery were uneventful and the development up to 7 months was good He received vaccines for diphtheria pertussis and tetanus three times polio live vaccine twice and B C G in the first week of life without any complication The child had not been seriously ill previously

He was sent to the hospital because of varicella with high fever of about 1 week s duration without any improvement at home The eruption at first maculo papulo vesicular became hemorrhagic and there were new crops appearing all over the body The high fever persisted and difficulty in breathin was noticed During the 5 days before admission he received Ampicillin and Erythromycin

On admission (14 9 69) severe hemorrhagic eruptions were noted over the trunk and limbs and especially crowded over the face The fever was 40 C and breathing was heavy Lymph nodes were palpated on the posterior neck A right purulent otitis media was found The physical findings of the lungs and heart were normal The edge of the liver was palpated but not the spleen

The child s condition became worse The high fever persisted and new skin lesions appeared Old lesions progressed in size and became confluent to larger areas like a third degree burn especially over the face (Fig 1) A severe enanthem and aphtic like ulcers appeared over the mucous surfaces of the lips tongue and pharynx X rays of chest after admission revealed interstitial bilateral d nsities pathognomonic of varicella pneumonia Another X ray examination after a week showed more severe densities in both lung fields

Laboratory findings

Hemoglobin 9 6 g/100 ml Hematocrit 30° W B C 4 200/mm³ with band forms 31 neutrophils 35 monocytes 2 and lymphocytes 32 Absolute count of lymphocytes was 1 340 A week before admission the W B C was 6 000 with 39 of lymphocytes which gives the absolute count of 2 340 lympho ytes Thrombocytes 88 000 and 48 000/mm³

Bloodgroup ARh – (isoagglutinins against erythrocytes group B present) Serological tests C F against mumps adenovirus Q fever negative Vaccinia HI < 5 vaccinia CF < 8 herpes simplex < 8 Varicella Zoster CF 16 (11th day after commencement of the eruption) Cerebro spinal fluid normal findings





Fig 3 Thymus Irregular islands of thymic tissue" surrounded by fibrous tissue H E × 1.0

Fig 4 Thymus No differentiation in cortex and medulla Spindle shaped cells with irregular distribution Scarce lymphocytes H E × 480

our patient who apparently was healthy before the varicella infection Vaccination with B C G after the birth and later with polio live vaccine did not cause any complications

The diagnosis of varicella appears also well established Two weeks before our patient fell ill with varicella the onset of varicella was diagnosed in his older sister by a physician and 14 days after taking the photo of our patient the assistant of the photographer of our hospital fell ill with typical varicella The primary lesions in our patient were typical For technical reasons the presence of varicella zoster (v z) virus in the lesion of the skin could not be demonstrated The positive CF (titre 16) for v z. virus found in the serum 11 days after commencement of the eruption however supports and the histological changes of the skin and other organs confirm the diagnosis of varicella

Secondary bacterial complications of varicella in children previously healthy are not infrequent (erysipelas cellulitis etc) More rare are complications directly connected with the v z virus encephalitis necrotic varicella thrombocytopenic purpura pneumonia (11 16 20) and nephritis (2 3 8 17 22) However death resulting from varicella is very rare perhaps with the exception in newborns in children treated with corticosteroids and immunosuppressive drugs (e g because of leukemia) and in adults with varicella pneumonia

On the other hand the great sensitivity to virus causing the death of children affected by thymic alymphoplasia is emphasized by various

Kidneys In sections with PAS staining there were some glomeruli which showed a thickening of the basal membrane and in the tubular epithelium some PAS positive granulation was found (hyaline droplet degeneration) We did not find necrosis and inclusion bodies and the present changes were interpreted as a membranous glomerulonephritis

Very important changes were noted in the lymphatic system

Thymus Small islands of altered thymic tissue were surrounded by large fibrous bands (Fig 3) In the so called thymic tissue there was no normal thymic parenchyma The difference between the structure of the cortex and the medulla was not evident, most of the parenchyma was built up of large spindle-shaped cells which showed a dark basophilic nucleus and an eosinophilic cytoplasm (Fig 4) In the centre of the island of thymic tissue there were some normal appearing lymphocytes Hassal bodies were scarce

Lymph nodes In some slides it was possible to distinguish between the cortex and the medulla, but nowhere did we find active germinal centres (Fig 5) and the pulpe was poor in lymphocytes Some proliferation of the reticulum cells was noted plasma cells were not found

Spleen The follicles were small and poor in lymphocytes (Fig 6)

Bone marrow Rich in cells normal erythroleukopoesis and presence of megakaryocytes No lymphocytes were found

Gastro intestinal tract In the mucosa scattered lymphocytes were found and very rare small lymphocytic accumulations which did not show the appearance of a follicle with an active germinal centre

In conclusion The changes in the skin and lungs are characteristic of varicella the membranous glomerulonephritis is a nonspecific inflammatory change

The profound alteration of the thymic structure and of the entire lymphatic system is compatible with the diagnosis of thymic alymphoplasia with dysgammaglobulinemia Remarkable is the absence of plasma cells in the lymph nodes and also in the other hematopoetic organs including the gastro intestinal tract



Fig 2 Skin Epidermal cell with intranuclear inclusion bodies surrounded by a clear halo H E ×480

COMMENTS

It appears certain that our patient suffered from thymic alymphoplasia with dysgammaglobulinemia (TAD) The low number of lymphocytes in the peripheral blood (1 340 and 2 340 in absolute count) the small amount of IgG and especially of IgM and the malignant course of the varicella infection resembling progressive vaccinia suggested clinically the diagnosis which was confirmed by autopsy

Generally infants affected by TAD suffer early in life from bacterial and even more from viral infections Cases are also known where such symptoms appear only several months after birth This was also the case with

tent We could not make investigations in this direction but the histological findings of the lungs after death are remarkable multiple necrotic areas were seen without inflammatory reaction as well as rare mononuclear infiltrates in the alveolar septa This was in contrast to the picture of varicella pneumonia of an im munologically normal child described by us (16) where numerous lymphocytes were found in the affected parts of the lungs

For the thrombocytopenic purpura observed in our patient two possibilities of explanation exist 1) It is possible that the thrombocytopenia without clinical signs was present before the varicella virus invasion and was aggravated by the infection 2) Another possibility is that the thrombocytopenia was directly caused by the actual virus infection The presence of normal megakaryocytes in the bone marrow favours the second explanation

As in other cases of TAD (12) the application of gammaglobulin and convalescent serum did not improve the clinical picture As emphasized by Burke & Skehel (1) antibodies are not the major factor in recovery from a viral infection since antibodies cannot penetrate cells and inhibit intracellular viral multiplication According to the same authors the reticuloendothelial system plays an important part in the production of interferon Possibly the abnormal stem-cell which is assumed to be the cause of TAD is also responsible for the incapacity of the reticuloendothelial system to produce normal interferon

SUMMARY

The authors present the case of a 7 month-old boy affected by thymic alymphoplasia with dysgammaglobulinemia who died from a severe varicella infection Clinical symptoms were hemorrhagic necrotic varicella pneumonia nephritis and continuous eruptions of fresh lesions for 14 days until death The lymphopenia the low content of gammaglobulin IgG and especially IgM in the serum of the child suggested the diagnosis of thymic alymphoplasia with dysgammaglobulinemia which was confirmed by autopsy Treatment with gammaglobulin and convalescent serum was without effect

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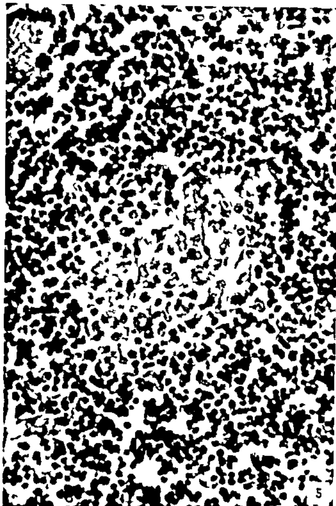


Fig 5 Lymph node The centre of a follicle built up of reticulum cells No mitotic activity H E ×240

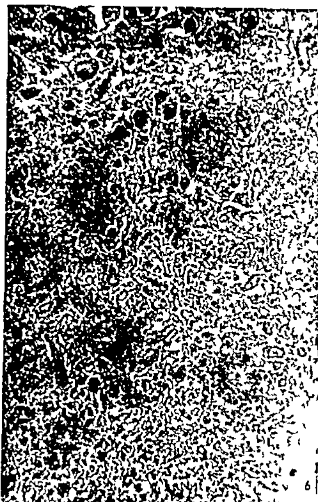


Fig 6 Spleen Rare small follicles Pulpa poor in lymphocytes H E ×120

authors especially to cytomegalic inclusion body virus to measles (14) and to vaccinia (13 18) Hoyer et al (12) described the death of 2 siblings caused by severe varicella and Fulginiti et al (5) another case who suffered from prolonged varicella

The continuous appearance of acute varicella lesions during the 17 days of illness and the progressive malignant course of the disease indicated the inability of the child to overcome the infection and induced us to investigate the possibility of an immunological defect

The gammaglobulin of the serum found by electrophoresis was very low (3 3% or absolute 135 3 mg/100 ml) The examination of the immunoglobulins revealed a fairly low content of IgG and a definitely low content of IgM These findings were especially conspicuous as in an immunologically normal child we would expect a rise in IgG and especially IgM during the 11 days of the varicella disease The absence of plasma cells in the lymph nodes and the other lymphoid tissues was in accordance with this dysgammaglobulinemia The presence of isoagglutinins against erythrocytes group B and the positive C F against v z virus (1 16) is of interest

The second significant finding was the low number of lymphocytes in the peripheral blood At the beginning of the infection the absolute number of lymphocytes was 2 340, but it fell later to 1 340 The number of lymphocytes in the peripheral blood of children affected by TAD is frequently low and the lymphocytes are probably also incompe

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CASE REPORT

POLIOMYELITIS ASSOCIATED WITH ORAL POLIOVACCINE

Report on Two Cases

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Trivalent oral poliovaccine (TOPV) of the Sabin types was introduced in Norway in the autumn of 1965 and soon replaced the inactivated poliovirus vaccine Through the health care program for infants and children TOPV is administered throughout the year Primary vaccination usually takes place in late infancy with three doses 4 to 6 weeks apart School children also have been vaccinated with TOPV, so that the majority of children in Norway have received this vaccine

From 1961 through 1965 an average of 24 cases of paralytic poliomyelitis have been reported annually During the following 5 year period only 8 cases have been notified altogether (8)

In the following will be described the clinical and virological findings in 2 children with paralytic poliomyelitis where the disease was associated with TOPV

CASE REPORTS

Case 1

Girl born Oct 18 1967 after uneventful pregnancy and delivery During the first 6 months of life she was hospitalized three times twice with vomiting and diarrhea and once with urinary tract infection From then on she suffered frequent upper respiratory tract infections, and was repeatedly treated with sulphonamides penicillin and ampicillin

DPT vaccination was done on June 4 and July 5 1968 She received TOPV first dose on Nov 5 1968 and 18 days later she would not use her left leg and reverted to crawling instead of walking with support By mistake she received a second dose of TOPV on Dec 4 1968 and that same day she was admitted to hospital

On admission 13 / months old she was somewhat irritable but in good nutritional shape Flaccid paralysis of the left lower leg was demonstrated both of the anterior and posterior muscle groups Ankle jerk was absent neurological findings otherwise were unremarkable Head circumference was 41 cm that is below the 2 5 percentile related to age

Hb 10 3 g/100 ml WBC 12 000/mm with left shift ESR 12 mm/hour Spinal fluid initially showed 10 WBC/mm³ later 3 WBC/mm and was chemically normal without bacterial growth Gammaglobulin analysis showed γG fraction to be 280 mg/100 ml which is somewhat low for her age γA 12 mg/100 ml and γM 20 mg/100 ml X rays of hips chest and skull were all normal Virological findings were as described below

On follow up at the age of 2 / years she still had paralysis with atrophy of the musculature of the left leg Her head circumference was small 43 2 cm She could speak only four or five single words Two months earlier she had started walking and she used her hands well making drawings Her psychomotor skills even though retarded had been improving over the last year She had not suffered serious infections lately

Case 2

Boy born Aug 15 1966 after uneventful pregnancy and delivery He had shown a normal psychomotor development At 4 months of age he suffered pneumonia and at the same time he had perianal abscess

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ths was not demonstrated in any of th se pa tients but they were both hospitalized some what late in the course of the disease The girl Case 1 was already microcephalic by the time she contracted paralytic poliomyelitis later signs of mental retardation were also noticed These findings are apparently unrelated to the paralytic disease

From Case 1 vaccine like poliovirus of both type 1 and type 2 were isolated and the serological findings were consistent with infection with both typ s During the acute phase of her illness however she was probably infected with poliovirus type 2 only as neutralizing anti bodies against type 2 and not against type 1 were demonstrated on the twelfth day of her illness

The boy Case 2 excreted a vaccine like poliovirus type 3 on several occasions Multiple infections would be less common among con tacts than among recipients of the trivalent oral vaccine Therefore infection with poliovirus type 3 only probably took place in this pa tient However antibodies against both poliovirus type 2 and type 3 were demonstrated and infection with poliovirus type 2 could not be excluded

Case 1 was directly associated and Case 2 indirectly associated with the use of TOPV Both cases fulfilled the criteria for vaccine as sociated cases of poliomyelitis set by a WHO Memorandum In recipients of vaccine those cases which occur within a month of vaccina tion and in contacts those which occur within 2 months of vaccination (6)

The 2 cases occurred within a period of 13 months and in two districts of Northern Nor way about 200 miles apart No other cases of poliomyelitis had been reported from this re gion of the country over the last 2 1/ years The epidemiological findings therefore did not suggest that any of the patients were in fected with an epidemic or "wild strain of poliovirus

Host factors may be of importance for the infection Vaccine associated paralytic poliomyelitis has been reported in a patient with hypogammaglobulinemia (3) Our Case 1 had recurrent infections of various types and the serum concentration of ,G globulin was found to be somewhat low which may possibly ex plain the adverse outcome of the infection with vaccine virus Case 2 had earlier pyogenic in fection but his serum gammaglobulins revealed values within normal limits

Paralytic poliomyelitis has become an ex tremely rare disease in Norway certainly due to the extensive immunization with trivalent oral poliovirus vaccine When a ca e does oc cur however a thorough virological as well as epidemiological investigation should be under taken in order to determine if the disease is caused by vaccine or naturally occurring poliovirus This must be done to assess the efficacy of the vaccination as well as the safety of the oral vaccine

SUMMARY

Two cases of paralytic poliomyelitis are re ported both associated with the use of trivalent oral poliovirus vaccine of the Sabin types The one a girl 13 1/ months old fell ill 18 days after she received the vaccine The other a boy nearly 3 1/ years old fell ill within 2 months after his elder brother was vaccinated The recipient case was probably due to type 2 the contact case probably due to type 3 vaccine virus

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Table I *Vaccine associated cases of paralytic poliomyelitis Virus isolations and serological findings*

Specimen	Day of illness	Result		
Case 1				
Spinal fluid	12	Negative		
Feces	13	Poliovirus types 1 and 2 vaccine like		
Feces	26	Poliovirus type 1 vaccine like		
Serum	12	<10	20	<10[a]
Serum	25	20	40	<10
Case 2				
Spinal fluid	18	Negative		
Throat washing	18	Negative		
Feces	18 24 31	Poliovirus type 3 vaccine like		
Feces	38 46 66 74	Negative		
Serum	19	<5	320	40[a]
Serum	25	<5	160	40

[a] Neutralizing antibodies against poliovirus type 1 2 and 3 in that order

He had been vaccinated against smallpox before 1 year of age otherwise he had not received any immunizations

A 10 year old brother received TOPV on Oct 29 and Nov 28 1969 On Dec 27 that year our patient fell ill with fever and pains in the neck and back lasting 1 week On the third day of this illness he started falling more often than he used to do the left leg seemed to be weak

On hospital admission Jan 14 1970 at 3 / years of age he was in excellent general condition but was limping Flaccid paralysis and some atrophy of the muscles of the left thigh was found with no demonstrable knee jerk Neurological findings otherwise appeared normal

WBC 5 800/mm^3 with normal differential count ESR 16 mm/hour Spinal fluid contained 6 WBC mm^3 all mononuclear protein was 25 mg/100 ml Gammaglobulin analysis revealed all three fractions well within normal limits Virological findings were as described below

One month after admission the boy was walking more steadily neurological findings however were unchanged

VIROLOGICAL FINDINGS

Specimens for virus isolation were inoculated into tubes of primary cultures of cynomolgus monkey kidney cells and human amnion cells and some of the fecal specimens were also injected intracerebrally into newborn mice In order to detect multiple virus infections virus positive specimens were also incubated with various combinations of hyperimmune sera against the three poliovirus types, before inoculation into cell culture tubes

The isolated poliovirus strains were tested for the following in vitro genetic markers Poliovirus type 1 in the r c t 39 3°C (reproductive capacity temperature 39 3°C) and the dextran sulphate sensitivity markers the poliovirus types 2 and 3 in the r c t 39 8°C marker and the intratypic serodifferentiation (Wecker) test (2 4 5 7)

Serum specimens were tested for neutralizing antibodies against the three poliovirus types using the Sabin strains as antigens

The results of the virus isolation attempts and the serological findings are summarized in the table

Fecal specimens from household contacts of both cases were also examined with negative results The specimens were however, collected late i e 7 weeks after the onset of the disease in Case 1, and 3 to 4 weeks after the disease started in Case 2

DISCUSSION

In most countries where live poliovirus vaccine has been administered on a large scale cases of vaccine associated paralytic poliomyelitis have been reported (6) The incidence of reported cases has varied probably due to several factors Variations in the intensity of investigating the disease may have been of major importance Other factors such as the immune status of the population and the composition of the oral vaccine have also contributed to the variations (1 6) All three types of poliovirus have been incriminated Since the autumn of 1965 when TOPV was introduced in Norway five million doses have been distributed and four or six vaccine associated cases have been notified including the 2 patients described in the present report (7)

In our 2 cases a clinical diagnosis of paralytic poliomyelitis was made Aseptic mening

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC ASSOCIATION

Meeting April 23 1971

G Berglund *What has happened to the leucemic children in Gothenburg since 1964*

L Engstrom P Karlberg & U Selstam *Birth weight and length in relation to gestational age*

A Gustafson I Kjellmer R Olegård & L Victorin *Elimination of intravenously administered fat in low birth weight infants*

The elimination rate of a single i v fat load of 0 5 g/kg b w (Intralipid®) was investigated in a group of LBW newborn infants In the pre term infants with birth weights appropriate for gestational age the fat was eliminated at a rate corresponding to that found in normal adults In light for-date infants however a slower elimination rate was found In this group of babies there also appeared a second generation of fat particles in the blood pre β lipoproteins A relation was found between the degree of light for-dateness and the retardation of fat disappearance

In another group of newborns Intralipid was administered during 8–12 hours at a rate of 0 15 g fat/kg per hour—corresponding to 3 6 g/kg and 24 hours The pre term infants appropriate for gestational age cleared their plasma and did not accumulate plasma lipids during the fat supply while the light for dates showed a progressive rise of the total plasma lipids of the pre β lipiproteins and of the chylomicrons In three light for-date infants heparin was given at eight hours and in all three a rapid drop in these plasma lipid fractions occurred

It is concluded that newborn infants appropriate for gestational age have a fat elimination capacity allowing the supply of 3–4 g fat/kg per 24 hours while light for-date infants have a considerably reduced elimination capacity

The study was supported by S mper Fond for Naringsforskning and Vitrum Ltd

A Hrbek T Olsson & P Karlberg *Evoked EEG responses in newborns with asphyxia and IRDS*

Two groups of premature and mature newborns were examined The first group included 13 infants with asphyxia of different degree the second group were 7 newborns with IRDS

Visual evoked responses (VER) to light flashes and somato-sensory responses (SsER) elicited by electrical pulses applied to n medianus were studied Also photic driving evoked by fast intermittent photostimulation was recorded

In all newborns with severe asphyxia (seven cases with Apgar score between 0 and 3) VER were distinctly altered Above all the initial fast components were affected The latency was in 6 of the 7 cases distinctly prolonged In moderate cases only in 2 from the 6 examined similar alterations were present All changes remained for a rather long time sometimes for weeks and even in infants without any clinical symptoms of cerebral damage

Photic driving was in asphyctic newborns worse than in normal infants

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Norway

Key words Oral poliovirus vaccine paralytic poliomyelitis vaccine complication

these sensitive strains to give asymptomatic bacteriuria or in some immunologic defect in these patients

In further attempts to study virulence factors of *E coli* bacteria the amounts of O and K antigens were measured in 15 O6 strains by the quantitative immunoelectrophoretic method of Laurell As standards were used isolated O6 antigen and K2a 2c and K13 antigens Because of the different electrical charge of the O and K antigen they could be measured simultaneously in the same run From the preliminary experiments there was a correlation between the amounts of O and K antigen and the resistance of the strains to the bactericidal effect of serum

Preliminary studies on Proteus bacteria causing UTI

While coliforms dominate among the bacteria causing UTI and is usually associated with acute infections *Proteus* is more frequently found in chronic infections and in patients with structural or functional urinary tract abnormalities These infection patterns might reflect differences in virulence between the two genera

For the clinically most important proteus species *P mirabilis* and *P vulgaris* a serological system comprising 49 O groups and 19 H groups has been worked out by Kauffmann & Perch On the basis of this serological system we tried to classify according to O group 107 proteus strains isolated from 76 children with UTI and also to correlate the O group to the clinical diagnosis For comparison were analyzed 117 strains isolated from 33 geriatric patients with UTI All strains from patients with significant bacteriuria were *O grouped* by agglutination with monovalent O antisera prepared in rabbits The method used was the same as earlier used for O grouping of *E coli*

With the 11 different O antisera available it was possible to classify strains from 47 of the 76 children The most common O groups were O_3 found in 10 patients O_{10} in 7 and O_{23} in 9 Other common O groups were O_{16} O_4 O_9 and O_{30} Analysis of the material according to diagnosis showed that the two patients with pyelonephritis both had O_3 bacteria In the 45 children considered to have cystitis and in the 8 patients where the available information was insufficient for a classification no specific O groups dominated Of 21 children with neurogenic bladder dysfunction 7 had O_3 strains

About two thirds of the 117 strains from the geriatric patients could be O grouped Approximately the same O groups were found in a rather similar frequency as in the pediatric material

Attempts to determine the antibody titer to *Proteus* in hyperimmune rabbit antisera using a homologous antigen as well as a pool consisting of eleven antigens with the passive hemagglutination method were successful The intention is now to study the antibody response in children with UTI caused by *Proteus* bacteria

Experiments with large pools of E coli O antigens for antibody titrations in patients with UTI

For studies of the antibody response to *E coli* O antigen in urinary tract infections (UTI) the bacteria isolated from the patient s urine are most p oper for preparation of antigen Andersen (1967) showed that a pool of the eight most common O antigens (8 pool) could be used for passive hemagglutination titrations This pool covers some 60% of the *E coli* strains most common in UTI

We have tried to enlarge the antigen pool to make it more useful in clinical work In preliminary experiments 12 different O antigens were used for coating the sheep red blood cells Most of them could be diluted about 50 times without a significant loss in titer Pools of 30 and 68 antigens were then compared with the 8-pool and with the antigen single in simultaneous titrations The large pools correlated well with each other but titers were lower than with single antigen or the 8 pool The 68 pool was chosen for titra

SsER were in newborns with severe asphyxia very small, but in contrast to VER they recovered rapidly

In all newborns with IRDS were VER during the first day of the disease altered Similar as in asphyctic newborns above all the initial components were affected The responses improved when oxygen supply was increased In contrast to asphyxias the responses became after several days quite normal (with one exception where the alterations remained for several weeks) Latency of VER was prolonged only in 2 of the 7 cases

In 3 newborns with IRDS simultaneously P_O was examined The behaviour of VER was always in accordance with blood oxygen tension

Our preliminary results seem to be encouraging Especially VER were distinctly altered in all severe asphyxias and in all IRDS cases during the acute state The changes were rather similar, but they remained much longer in infants after asphyxia Continuation of the study will show in what degree the methods can be used for testing of the brain functions during the disease and if they may provide some prognostic data for the further development

U Selstam P Karlberg & T Landstrom *Experiences with notifications of medical birth records*

L A Hanson G Fridh J Holmgren U Jodal B Kaijser P Larsson J Melchior & S Olling *Urinary tract infections (UTI) in infants and children further bacteriological immunological and clinical studies*

The high prevalence of UTI the high risk of recurrence and also of developing renal damage makes it an infectious disease of great clinical concern Most of the cases appear in children without any known obstruction or other known cause of the infection Therefore we have been interested in studies of factors that can be of importance for the appearance and course of these infections This panel presents investigations of some characteristics of the bacteria involved the immune response to these bacteria and finally the possible mechanisms of the pyelonephritic renal damage

Studies of possible virulence factors in E coli strains causing UTI

In order to test if resistance to the bactericidal action of normal serum is a virulence factor for bacteria causing urinary tract infection 450 *E coli* strains isolated from urine blood or feces were tested After incubation of each strain for 30 min with complement containing sera from five healthy adults the percent surviving bacteria was determined The five sera gave very similar results and the five tests for each strain were therefore combined and the strains were designated resistant or sensitive to the bactericidal activity of blood serum It was found that about 70% of the smooth coli strains were resistant, with no significant differences between the strains from urine, blood or feces In contrast the rough strains were sensitive in a much higher frequency

Strains of O groups commonly isolated from patients with UTI (e g O groups 4 6 and 18) were more often resistant (in 90–95%) than strains of O groups uncommon in UTI not typable strains or rough strains (in 10–20%) These findings suggest that resistance to the bactericidal action of normal serum might contribute to the prevalence of strains of certain O groups in feces i e the same strains that are commonly found as cause of UTI since the stool often seems to be the source of the infecting strain in UTI As yet there is no definite evidence however that only the most resistant fecal strains can invade the blood or the urinary tract

No difference was observed in resistance to serum bactericidal activity between strains from 54 patients with cystitis pyelonephritis or insignificant bacteriuria However only two of the eight strains from eight patients with asymptomatic bacteriuria were resistant It is too early to speculate in a special property of

virulent bacteria and/or a defect in host de fence could lead to severe infections causing irreversible renal damage As yet however neither especially nephritogenic *E coli* strains nor any general or local immune defect has been recognized that could explain the appearance of the renal damage in patients who have no structural or functional abnorma lities in their urinary tracts Furthermore from studies of patients as well as experi mental animals evidence has accumulated that the kidney lesions can progress in spite of there being no demonstrable infection We are therefore at present more tempted to suspect that the immune response to the in fecting bacteria might be involved in the pathogenesis of the renal lesions

Bacterial endotoxin is present in the kidney for a long time after the infection and might initiate the kidney damage by activating the complement system after combination with antibody or perhaps even without the help of the antibody Another possible mechanism for the development of the renal lesions was re cently suggested by our finding that antisera against certain *E coli* strains could react with antigen prepared from normal human kidney (Holmgren Hanson Holm & Kaijser *Int Arch Allergy* in press) This observation could be the first step in a chain leading to a classifica tion of pyelonephritic renal lesions as the result of an autoimmune process induced by crossreacting bacterial and renal antigens

Johan Gentz

tions with sequences of sera from 26 children with acute febrile pyelonephritis caused by *E coli* All had elevated sedimentation rate and decreased concentrating capacity as indications of parenchymal engagement Both the 8- and especially the 68 pool showed a considerable scatter of titers when compared with the single antigen prepared from the infecting strain Using the highest value in each sequence 21 11 and 3 significantly elevated titers out of 26 were found using the homologous antigen, the 8 pool and the 68 pool respectively However these numbers were increased to 21 18 and 7 when the sequences of sera were analysed and changes of at least three titer steps were considered significant to indicate an actual antibody response The 8 pool thus is of value for these titrations if sequences of sera are used whereas the large pool means a considerable decrease in frequency of significantly elevated titers A more attractive way of covering a larger proportion of the *E coli* strains seen in UTI should be an antigen common for all these strains and of such a nature that it could be used both for passive hemagglutination and precipitation

Antibodies to K antigen and a common protein antigen in children with UTI caused by E coli O6

Most studies concerning antibodies in patients with urinary tract infections have till now only dealt with O antibodies Since K antigen is said to be a virulence factor by diminishing the susceptibility of the bacteria to complement and phagocytosis the antibody response to this antigen should be of interest to study We have isolated the K antigen from *E coli* O6 K2a 2c H1 and O6 K13 H1 and studied by passive hemagglutination the K antibody response in 32 children with UTI caused by *E coli* O6 bacteria

In most cases with pyelonephritis caused by *E coli* O6 K13 or O6 K2a 2c there was a K antibody response almost parallel to the O antibody response but with lower titer values In three patients, however no rise in K antibody titers was found in spite of an ordinary O antibody response This could possibly indicate a defect in the antibody response to the K antigen in these patients No patients with cystitis showed O or K antibodies

From earlier studies we know that *E coli* bacteria have at least around 20 different antigens We also know that some of these antigens are common to many *E coli* bacteria irrespective of O groups Recent studies by comparative immunoelectrophoresis of 11 different *E coli* strains showed that at least one distinct antigen is common to these strains This antigen showed a negative electric charge and a high electrophoretic mobility It was possible to isolate it from *E coli* O18 K76 H14 and O4 K3 H5 by preparative electrophoresis and it was shown to be heat labile and digestible by a pronase Most probably it is of protein nature Determination by Molisch's reaction, however, indicated that it also contains carbohydrate

An antibody response to this common protein antigen was shown by passive hemagglutination in 5 children with pyelonephritis caused by *E coli* O6 The titer rise was almost parallel to the O antibody response but lower titer values were obtained No antibody response was detected in children with cystitis

This common protein antigen might perhaps be used for detection of an antibody response to *E coli* of many perhaps all O groups It might be used in passive hemagglutination instead of the patients own strain and instead of large *E coli* pools

On the pathogenesis of the pyelonephritic renal lesions

The mechanism behind the parenchymal reduction appearing in some patients with pyelonephritis is largely unknown The infecting bacteria in the majority of the cases belonging to the *Escherichia* genus as well as the host response to the infecting microorganisms should be considered in a discussion of the pathogenesis of these lesions Thus especially

virulent bacteria and/or a defect in host defence could lead to severe infections causing irreversible renal damage As yet however neither especially nephritogenic *E coli* strains nor any general or local immune defect has been recognized that could explain the appearance of the renal damage in patients who have no structural or functional abnormalities in their urinary tracts Furthermore from studies of patients as well as experimental animals evidence has accumulated that the kidney lesions can progress in spite of there being no demonstrable infection We are therefore at present more tempted to suspect that the immune response to the infecting bacteria might be involved in the pathogenesis of the renal lesions

Bacterial endotoxin is present in the kidney for a long time after the infection and might initiate the kidney damage by activating the complement system after combination with antibody or perhaps even without the help of the antibody Another possible mechanism for the development of the renal lesions was recently suggested by our finding that antisera against certain *E coli* strains could react with antigen prepared from normal human kidney (Holmgren Hanson Holm & Kaijser *Int Arch Allergy* in press) This observation could be the first step in a chain leading to a classification of pyelonephritic renal lesions as the result of an autoimmune process induced by crossreacting bacterial and renal antigens

Johan Gentz

NEW BOOKS RECEIVED

The prevention of perinatal mortality World Health Organization Technical Report Series No 457 Geneva 1970

Endocrine regulation of human gestation World Health Organization Technical Report Series No 471 Geneva 1971

V Apgar (ed) *Downs syndrome (Mongolism)* 386 pp illus The New York Academy of Sciences New York 1970 US $24 50

H Behrendt & M Green *Patterns of skin pH from birth through adolescence With a synopsis on skin growth* 115 pp illus Charles C Thomas Publisher Springfield 1971 US $8 25

J P Colombo *Congenital disorders of the urea cycle and ammonia detoxication* 150 pp illus S Karger Munchen Paris London New York and Sydney 1971 DM 51 —

I Gamstorp *Pediatric neurology* 394 pp illus Butterworths London 1970 Price not given

T Gedde Dahl Jr *Epidermolysis Bullosa* 180 pp illus Universitetsforlaget Oslo 1971 Nkr 120 —

E Goettsch *Kidney disease in the young* 305 pp illus W B Saunders Company Ltd London 1971 £9 60

M Ingle Wright *The pathology of deafness An introduction* 178 pp The Williams & Wilkins Company Manchester 1971 £2 64

B Ivemark *Barnpatologi Vägar till diagnos* 332 pp illus Almqvist & Wiksell Stockholm 1971 Price not given

W Th Koos & M H Miller *Intracranial tumors of infants and children* 415 pp illus Georg Thieme Verlag Stuttgart 1971 DM 130 —

J Lang *Strabismus Diagnostik Schielformen Therapie* 206 pp illus Hans Huber Bern 1971 Fr 28 —

R Lightwood F Brimblecombe & D Barltrop *Paterson's sick children* 795 pp illus Bailliere Tindall & Cassell Ltd London 1971 95s

J L Melnick (ed) *Progress in medical virology* Vol 13 503 pp illus S Karger Basel Munchen Paris London New York and Sydney 1971 US $26 40

J Muller *Regulation of aldosterone biosynthesis* 137 pp illus Springer Verlag Berlin 1970 DM 36 —

D Palitzsch *Systematik der praktischen Pädiatrie fur Ärzte und Studenten Daten Fakten Übersichten* 435 pp illus Georg Thieme Verlag, Stuttgart 1971 DM 24 80

G Polgar & V Promadhat *Pulmonary function testing in children* 273 pp illus W B Saunders Company Ltd London 1971 £9 15

J J G Prick *Infantile autistic behaviour and experience A new clinical picture* 100 pp illus Rotterdam University Press Rotterdam 1971 D Fl 35 —

E Rossi (ed) *Pädiatrische Gastroenterologie* 135 pp illus S Karger Basel Munchen Paris London New York and Sydney 1971 US $4 30

W J W Sharrard *Paediatric orthopaedics and fractures* 1126 pp illus Blackwell Scientific Publications Oxford 1971 £15 —

B C L Touwen & H F R Prechtl *The neurological examination of the child with minor nervous dysfunction* 105 pp illus William Heinemann Medical Books Ltd London 1970 £2 25

P H Wender *Minimal brain dysfunction in children* 242 pp illus John Wiley & Sons Ltd Chichester 1971 £5

J V van Wieringen F Wafelbakker H P Verbrugge & J H de Haas *Growth diagrams 1965 Netherlands* 68 pp illus Wolters Noordhoff Publishing Groningen 1971 Price not given

R Andre & R Gyselings *La mortalie infantile en Belgique* 169 pp illus Éditions de l Institut de Sociologie Université Libre de Bruxelles Bruxelles 1971 Price not given

E D Acheson (sc ed) *Epidemiology of non-communicable disease* British Medical Bulletin vol 27 1971 London £2

R J K Brown & H B Valman *Practical neonatal paediatrics* 92 pp illus Blackwell Scientific Publications Oxford 1971 £1 25

P Benson (ed) *The biochemistry of development* Clinics in Developmental Medicine No 37 273 pp illus Spastics International Medical Publications William Heinemann Medical Books Ltd London 1971 £3 25

R H Gevers & J H Ruys (eds) *Physiology and pathology in the perinatal period* 199 pp illus Leiden University Press Leiden 1971 D Fl 36 —

E Góth & Fovényi (eds) *Polypeptide hormones* Proceedings of the Fourth Congress of The Hungarian Society of Endocrinology and Metabolism Sept 29 – Oct 1 Budapest 1969 492 pp illus Akademiai Kiado Budapest 1971 US $17 00

G A von Harnack (ed) *Kinderheilkunde* 453 pp illus Springer Verlag Berlin Heidelberg and New York 1971 DM 36 —

U Kottgen B K Jungst W Toussaint & P Emmrich (eds) *Padiatrische Intensivpflege* Bericht uber das 1 Symposion vom 17 und 18 April Mainz 1970 199 pp illus Ferdinand Enke Verlag Stuttgart 1971 DM 48 —

J-C Lafon (ed) *La Surdite du Premier Age* Colloque International d Audiophonologie les 9 10 11 12 novembre Besançon 1969 51 pp illus Editions Camponovo 25 Besançon 1971 Price not given

R W A Oliver & S A Oliver *The analysis of childrens urine An annotated and cross referenced bibliography* 112 pp Heyden & Son Ltd London 1971 Part 1 £3 75 Part 2 £4 25

E Rossi (ed) *Hamatologie im Kindesalter Paediatrische Fortbildungskurse fur die Praxis* vol 31 125 pp illus S Karger Basel Munchen Paris London New York and Sydney 1971 sFr 35 —

E Schenk *Neurologische Untersuchungsmethoden* 252 pp illus Georg Thieme Verlag Stuttgart 1971 DM 13 80

ANNOUNCEMENTS

The *Fundación* Viviana Luckhaus has instituted the International Prize Fundacion Viviana Luckhaus 1972

The Prize is intended to honour a report of original research related to blood platelets (morphology physiology biochemistry pathology etc) and/or their relationship to thrombosis and blood vessels and to promote communication and interchange between research workers in different parts of the world

For further information and rules for the 1972 contest apply to Dr Edgardo S Sack Fundación Viviana Luckhaus Hospital Juan A Fernandez Cervino 3356 Buenos Aires Argentina

The 3rd European Congress on Pediatric Neurosurgery planned to take place in Gottingen from 21st to 23rd September 1972 will be held from 3rd to 7th September 1972 Subjects

1 Problems of anaesthesia and postoperative control of neurosurgical baby and infant patients
2 Longterm catamnesis after neurosurgical intervention in babies and infants
3 Neuroradiology of babies and infants
4 Recent diagnostic and operative techniques in pediatric neurosurgery
5 Demonstrations of rare cases
6 Self-chosen subjects

Applications to present a paper should be received not later than Nov 1st 1971 For information please write to the chairman Prof Dr med K A Bushe Direktor der Neurochirurgischen Klinik der Universitat Gottingen 3400 Gottingen Gosslerstraße 10

The International Prize for Modern Nutrition amounting to 15 000 S fr will be awarded in 1972 for recent research work on the subject *Malnutrition and its gastrointestinal and metabolic consequences*

The International Prize for Modern Nutrition is offered to persons who have made significant contributions to the following subject

Scientific work on the effects of malnutrition (Kwashiorkhor and Marasmus) on gastrointestinal and metabolic functions Emphasis should lie on work concerning specific effects in particular enzymatic deficiencies of the small intestine liver and pancreas Furthermore work concerning the metabolic consequences of malnutrition including disturbances of the endocrine system will also be considered

Information Professor Dr M D mole Service de Dietetique Hopital Cantonal CH 1211 Geneve 4

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CEREBROSPINAL FLUID LACTATE/PYRUVATE RATIO IN NORMAL AND ASPHYXIATED NEONATES

N W SVENNINGSEN and B K SIESJÖ

From the Department of Paediatrics and the Brain Research Laboratory E blocket University Hospital of Lund Sweden

Since the 1940s supplemental oxygen has been used in the care of asphyxiated neonates However the potential hazards of excess oxygen administration such as retrolental fibroplasia (24 25) and diffuse pulmonary dysplasia (19 22) have made it desirable to develop suitable methods for the detection and quantitation of hypoxia Under clinical circumstances apart from the registration of crude signs such as cyanosis and respiratory changes (32) measurement of arterial P_O oxygen saturation and acid-base status (15 17 23) and of the blood lactate content (4 6 10) and even measurement of the subcutaneous P_O (14) have been used to evaluate the degree of asphyxia and oxygen deficiency However these methods hitherto used fail to register the degree of cerebral hypoxia which is an important determinant of the immediate survival as well as the long term prognosis of the neonates

It has been pointed out that since lactate and pyruvate are freely diffusible through most cell membranes and since the lactate/pyruvate system is coupled in an equilibrium reaction to the cytoplasmatic NADH/NAD$^+$ system (11 12 13) measurements of extracellular lactate/pyruvate ratios can be useful for detecting tissue hypoxia The lactate/pyruvate ratio in the cerebrospinal fluid (CSF) seems to reflect changes in the cerebral NADH/NAD$^+$ system both in various forms of experimental cerebral hypoxia (8 16 28) and in clinical cases in which cerebral hypoxia may be assumed to be present (9 26)

The present report is a preliminary account of the CSF lactate/pyruvate ratio in normal and a number of asphyxiated newborn infants Arterial P_O and acid-base variables in arterial blood and CSF were also measured

MATERIAL

This investigation comprises a total of 40 neonates who were studied at the postnatal age of 3 hours to 25 days

Neonates without clinical signs of asphyxia (Group I)

19 newborn infants were investigated with paired samples of *simultaneously taken* arterial (umbilical artery or radial artery) blood samples and lumbar CSF samples The clinical data of these 19 neonates are presented in Table 1 These infants passed through the gestational and perinatal period without showing any clinical signs of asphyxia

Neonates with clinical signs of asphyxia (Group II)

The 21 asphyxiated neonates in this group were studied in a similar manner with *simultaneously taken* arterial blood and lumbar CSF samples Table 2 gives the clinical data of these 21 infants including abnormalities during delivery and the type of asphyxia sustained in the perinatal period In the following presentation the results in group II are divided into two categories according to the interval between the asphyxial incident and the sampling of arterial blood and lumbar CSF i e interval less than 24 hours = Group II A (14 *infants* and 21 simultaneously taken samples) and the interval *more than* 24 hours = Group II B (7 infants and 9 simultaneously taken samples)

Table 1 *Clinical data of 19 neonates without perinatal asphyxia, i e normal control group (Group I)*

Case No	Birth weight (g)	Gestational age (weeks)	Apgar score (1–10 min)	Neonatal period
1	2 530	35	10–10	Uneventful
2	2 350	34	10–10	Uneventful
3	2 790	38	10–10	Uneventful
4	2 260	32	8–10	Uneventful
5	2 710	38	8–10	Uneventful
6	3 840	40	10–10	Urinary tract infection
7	1 700	33	9–10	Duplex I
8	4 010	40	10–10	VOC cong ?
9	3 200	37	8–10	Urinary tract infection
10	2 740	38	10–10	Rh immunization
11	3 170	40	9–10	Hyperbilirubinemia
12	4 100	40	10–10	Pemphigus neonatorum
13	3 100	40	10–10	Feeding difficulties
14	4 130	40	10–10	VOC cong (septal defect)
15	3 020	39	10 10	VOC cong (septal defect)
16	2 150	37	9–10	Mb Down
17	3 500	40	9–10	Duplex I
18	2 700	39	10–10	Hyperbilirubinemia
19	3 300	40	10–10	Cleft lip

The clinical criteria of perinatal asphyxia in this study were Fetal heart rate dropping below 20 beats per 15 sec (intrauterine asphyxia) *and/or* Apgar score below 6 points at 10 min after delivery (including heart rate below 100 beats per minute) *and/or* cyanotic attacks or apnoic spells with heart rate decreasing below 100 beats per minute

Blood and CSF collection

Arterial blood (umbilical artery or radial artery) was taken anaerobically and pH P_{CO_2} and P_{O_2} of the arterial blood samples were measured within 15 min The CSF samples were collected anaerobically by lumbar puncture 2 ml in a glass syringe and were analysed within 15 min for pH and P_{CO_2} A portion (0 5 ml) of the CSF sample was kept at −85 C for later analysis of lactate and pyruvate All determinations were made in duplicate

The arterial blood and CSF samples were collected without any supplemental oxygen being administered The babies were nursed either in an Isolette Incubator or an AGA Infant Incubator and the percentage of oxygen in the environment was measured by a Fieldlab Oxygen Analyzer

METHODS

pH P_{CO_2} and P_{O_2} were measured at 37 C using microelectrodes (Eschweiler & Co Kiel) The pH and blood gas values were corrected for the actual body temperature

Table 2 *Clinical data of 21 neonates with neonatal asphyxia (Group II)*

Case no	Birth weight (g)	Gestational age (weeks)	Delivery	Apgar score (1–10 min)	Neonatal period
IIA 1 a	2 150	36	Breech delivery	2– 8	IRDS and apnoic spells
IIA 2 a	3 900	42	Prolonged labour (48 hours)	3– 8	Apnoic spells convulsions
IIB 3 a	1 900	34	Breech delivery Forceps	4– 9	Cyanotic attacks
IIA 4 a	2 060	32	Duplex II	8–10	Septicemia Apnoic spells Died
IIB 5 a	2 400	34	Intra uterine asphyxia	3– 8	IRDS and apnoic spells
IIA 6 a	3 110	40	Intra uterine asphyxia	4– 6	Postnatal asphyxia Apnoic spells
IIA 7 a	2 780	41	Uneventful	9–10	Cyanotic attacks
IIA 8 a	3 830	41	Forceps	4– 8	Cyanotic attacks
IIB 9 a	3 760	42	Intra uterine asphyxia	3– 7	Apnoic spells Hypoglycemia
IIA 10 a	2 480	36	Cesarian section	5–10	Diabetic fethopathy Hypoglycemia
IIA 11 a	3 660	39	Uneventful	7– 9	Cyanotic attacks Hypotonia
IIA 12 a	1 700	31	Uneventful	8–10	Cyanosis Apnoic spells
IIA 13 a	2 380	39	Uneventful	8–10	Small for gestational age Cyanotic attacks
IIB 14 a	2 150	38	Intra uterine asphyxia	8–10	Small for gestational age Jitteriness
IIB 15 a	3 700	40	Prolonged labour (72 hours)	4–10	Apnoic spells
IIB 16 a	3 120	40	Intra uterine asphyxia	3–10	Hypotonia Cyanosis
IIB 17 a	3 080	40	Forceps	2–10	Cyanotic attacks
IIA 18 a	2 190	38	Uneventful	8–10	Small for gestational age Cyanotic attacks
IIA 19 a	1 360	30	Ablatio placentae	7–10	Respiratory insufficiency syndrome Respirator treatment
IIA 20 a	2 420	36	Uneventful	7–10	Cyanotic attacks
IIA 21 a	2 190	35	Breech delivery	8–10	Cyanotic attacks

Table 3 *Mean values standard deviations (S D) and ranges of acid-base variables in arterial blood and cerebrospinal fluid (CSF) in simultaneously taken samples from 40 newborn infants*

Ns = Number of samples analysed *p* value = Probability estimation comparing group I with group II A and group II B respectively

	Arterial blood				CSF		
	Po (mmHg)	Pco (mmHg)	pH	Base excess (mEq/l)	Pco (mmHg)	pH	Bicarbonate (mEq/l)
Group I (Control infants) (*Ns* = 24)							
Mean	64 3	39 9	7 356	−3 3	49 3	7 331	24 5
S D	±9 6	±4 7	±0 035	±2 2	±7 8	±0 037	±4 2
Range	35 5–77 0	30 0–50 0	7 270–7 400	−6 7– −1 0	38 0–63 0	7 280–7 460	16 9–31 7
Group II (Asphyxiated infants)							
Group II A (sampling within 24 hours) (*Ns* = 21)							
Mean	49 5	40 3	7 367	−4 5	48 1	7 323	23 4
S D	±9 3	±7 3	±0 065	±3 9	±9 9	±0 044	±4 1
Range	28 0–63 0	28 5 56 0	7 225–7 480	−10 8 +6 0	32 5–67 0	7 270–7 395	14 6–33 2
p	<0 001	>0 35	>0 [illegible]	>0 10	>0 30	>0 20	>0 10
Group II B (sampling after 24 hours) (*Ns* = 9)							
Mean	71 9	36 0	7 390	−3 0	41 4	7 344	23 3
S D	±9 4	±6 6	±0 047	±3 9	±10 4	±0 037	±3 2
Range	48 0–88 0	24 0–47 5	7 300–7 465	−8 8– +3 6	23 0–54 0	7 310–7 440	18 5–26 5
p	>0 01	>0 01	>0 01	>0 35	>0 01	>0 10	>0 20

(27) The whole blood base excess value was calculated from the pH the Pco and the hemoglobin concentration using the Siggaard Andersen alignment nomogram (31) The CSF bicarbonate concentration was calculated using pK for carbonic acid of 6 125 and a CO_2 solubility f 0 0314 mMol/l mmHg

The CSF lactate and pyruvate concentrations were measured by enzymatic procedures after extraction with a perchloric acid neutralisation of the extract to pH 5 with 5N KOH and subsequent centrifugation (11) The measurements were performed on a Zeiss PMQ II spectrophotometer with recording of each enzymatic curve (8)

RESULTS

Acid–base variables (Table 3)

Arterial blood In the control group (Group I) without clinical signs of asphyxia the mean values and standard deviations were for pH 7 356 ±0 035 for P_{co} 39 9±4 7 mmHg for base excess −3 3±2 2 mEq/l and for P_o 64 3±9 6 mmHg As shown in Table 3 these values in the asphyxiated infants did not differ significantly from the values found in the control group It must be pointed out that in all our cases of asphyxia in group II initial arterial blood acid–base variables registered within the first hour after the asphyxial incident showed different degrees of acidosis in the blood However when the *simultaneously taken* arterial blood and CSF samples were obtained (in group II A in average 8 19 hours (range 1 to 18 hours) and in group II B more than 24 hours after the asphyxial incident) the arterial blood acid–base values had become normal in most cases as is evident from the mean and range values in Table 3 Because of this interval the acid–base values of the asphyxiated infants as presented in Table 3 are mainly within the normal range

When the arterial acid–base and blood gas values in the normal and the asphyxiated infants were compared the only difference to be found was the arterial P_o level being significantly lower in group II A as compared to the control group of normal neonates ($p<0\ 001$) It is well known that in the normal newborn infant during the first week of life there is a delayed adjustment of arterial P_o to normal adult values (17 18) which is also apparent from the P_o values obtained in this study

Cerebrospinal fluid (CSF) In our control group (Group I) of non asphyxiated neonates the mean values the standard deviations and the ranges

Table 4 *Mean values, standard deviations (S D) and ranges of lactate and pyruvate concentrations and the lactate/pyruvate ratios in cerebrospinal fluid (CSF) of 40 newborn infants*

Ns = Number of samples analysed *p* value = Probability estimation comparing group I with group II A and group II B respectively

	Lactate (mMol/l)	Pyruvate (mMol/l)	Lactate/pyruvate ratio
Group I Control infants (Ns = 24)			
Mean	1 601	0 098	16 5
S D	±0 322	±0 017	±0 8
Range	1 046–2 262	0 068–0 142	14 3–17 6
Group II Asphyxiated infants			
Group II A (sampling within 24 hours) (*Ns* = 21)			
Mean	2 081	0 105	19 8
S D	±0 694	±0 028	±2 8
Range	1 395–4 416	0 071–0 182	17 8–28 1
p	<0 001	>0 1	<0 001
II B (sampling after 24 hours) (*Ns* = 9)			
Mean	1 712	0 112	15 6
S D	±0 381	±0 036	±1 7
Range	1 261–2 493	0 078–0 204	12 2–17 5
p	>0 2	>0 05	>0 02

were for pH 7 331 ± 0 037 with a range of 7 280 to 7 460, for P_{CO} 49 3 ± 7 8 mmHg with a range of 38 0 to 63 0 mmHg and for bicarbonate 24 5 ± 4 2 mEq/l with a range of 16 9 to 31 7 mEq/l *These values are comparable to findings* recorded in older infants by other investigators (1)

The CSF P_{CO} was higher than the arterial P_{CO} This difference is in accordance with observations made on adults (3) In the asphyxiated infants the values for CSF pH P_{CO} and bicarbonate did not differ significantly from those in the normal control group Even here the interval as previously mentioned might be responsible for the lack of any significant difference between the normal and the asphyxiated infants

Lactate and pyruvate in CSF

Normal neonates (Group I) Table 4 presents the mean values the standard deviations and the ranges of the CSF lactate and pyruvate concentrations as well as of the CSF lactate/pyruvate ratios In the control group of 19 neonates without perinatal asphyxia (Group I) the means and standard deviations were for *lactate* 1 601 ± 0 322 mMol/l for *pyruvate* 0 098 ± 0 017 mMol/l and for *lactate/pyruvate ratio* 16 5 ± 0 8 Although a tendency to higher values was observed during the first 24 hours of life for both lactate and pyruvate (Figs 1, 2) it is noteworthy that the values were widely scattered within a large range This is in contrast to the lactate/pyruvate ratios which were uniformly distributed within a narrow range i e 14 3 to 17 6 during the whole neonatal period until 25 days of postnatal age (Fig 3)

Asphyxiated neonates (Group II) Table 4 also presents the CSF lactate and pyruvate concentrations and the lactate/pyruvate ratios for the infants who had sustained asphyxia The *CSF lactate* concentration was in group II A 2 081 ± 0 694 mMol/l and in group II B 1 712 ± 0 381 mMol/l The *CSF pyruvate* concentration was in group II A 0 105 ± 0 028 mMol/l and in group II B 0 112 ± 0 036 mMol/l Thus the mean lactate and pyruvate concentrations were somewhat

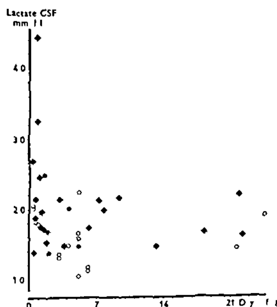


Fig 1 CSF lactate concentration in relation to postnatal age Normal neonates i e group I ○ (*N* = 19 Number of samples = 24) Asphyxiated neonates i e group II (*N* = 21 Number of samples = 30) subdivided into two groups according to the time interval between the asphyxial incident and CSF sampling either less than 24 hours i e group II A ◆ (*N* = 14 Number of samples = 21) or more than 24 hours i e group II B ● (*N* = 7 Number of samples = 9)

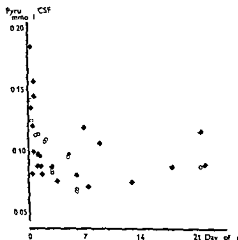


Fig 2 CSF pyruvate concentration in relation to post natal age Symbols as in Fig 1

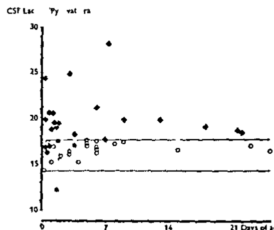


Fig 3 CSF lactate/pyruvate ratio in relation to postnatal age The dotted lines indicate the range observed in normal neonates Symbols as in Figs 1 and 2

higher in the two groups of asphyxiated neonates than in the control group but only the increase in the CSF lactate concentration in group II A was statistically significant ($p<0\,001$) in com parison with group I However the single values in all groups were widely scattered and as is evident in Figs 1 and 2 they fell mainly within the same range for all three groups of newborn infants

The *CSF lactate/pyruvate ratio* in group II A was significantly higher (mean value $19\,8\pm2\,8$ with range 17 8 to 28 1) than in the control group (Group I) and in group II B ($p<0\,001$)

High lactate/pyruvate ratios were found in group II A throughout the neonatal period with the exception of three cases (Fig 3) These three infants (case 18*a* 20*a* and 21*a* in Table 2) had sudden attacks of cyanosis with bradycardia at a postnatal age of 20 12 and 11 hours respec tively Arterial acid–base status taken 15 to 20 min after the cyanotic attack showed mode rate acidosis (pH 7 24 7 18 7 15 and P_{CO} 42 0 50 0 45 5 mmHg respectively) whereas the lowest arterial P_O values (umbilical artery) registered were for each 61 64 and 63 mmHg Thus these values of arterial P_O were within the normal range at this postnatal age (18) In addition to this the CSF lactate/pyruvate ratios studied 10 11 and 12 hours respectively after the asphyxial incident were within the range of the nonasphyxiated neonates as shown in Fig 3 This may implicate that these three infants although showing clinical signs of asphyxia had not sustained any cerebral hypoxia

The relationship between the CSF lactate/ pyruvate ratio and the interval between the asphyxial incident and the CSF sampling is pre sented in Fig 4 This figure shows the higher lactate/pyruvate ratios found in infants studied

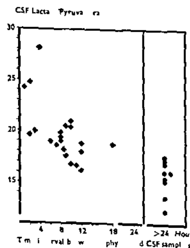


Fig 4 Relationship between the CSF lactate/pyruvate ratio in asphyxiated neonates *and* the *time interval* between the asphyxial incident and the CSF sampling. ◆ Group IIA ● Group II B

within 24 hours of asphyxia, the only exceptions being the three cases (cases 18*a*, 20*a* and 21*a*) mentioned above In the infants who were followed with repeated CSF sampling the lactate/pyruvate ratios decreased, and when repeated later than 24 hours after the asphyxial incident these values fell within the normal range

DISCUSSION

The present results have shown that the CSF lactate/pyruvate ratios were increased in the majority of neonates with clinical symptoms of asphyxia who were studied within 24 hours of the asphyxia incident A marked increase was found in the immediate post asphyctic period whereas the lactate/pyruvate ratio was normal in all cases studied later than 24 hours after the asphyxia (Fig 4) In many cases the increased lactate/pyruvate ratios were associated with high lactate concentrations but the lactate (and the pyruvate) concentrations varied too much both in the control group and in the asphyctic group to reveal any significant correlation between the symptomatology and the lactate levels

As pointed out in the introduction there is reason to believe that the CSF lactate/pyruvate ratio reflects the redox state of the cytoplasmatic $NADH/NAD^+$ system in brain cells and that cerebral hypoxia should be accompanied by increases in the CSF lactate/pyruvate ratio It is generally assumed that lactate and pyruvate pass but slowly from the blood plasma to the CSF (2, 20) and therefore changes in the blood lactate and pyruvate concentrations have relatively little influence on the corresponding CSF concentrations However, recent results have indicated that a faster flux of lactate and pyruvate may occur in arterial hypoxemia and in hypercapnia (21 30) and it remains to be shown that the present results may not be explained at least partly, by an influence from (unrecorded) blood lactate and pyruvate changes

Although the results should be interpreted with caution it seems likely that the CSF lactate/pyruvate changes reflect cerebral events and that they are secondary to tissue hypoxia In tissue acidosis caused by e g hypercapnia the lactate/pyruvate ratio may also increase (8 29) Hypercapnia cannot however, explain the present results, since the arterial P_{CO} registered in our cases was not increased and there was no correlation between the CSF CO_2 tension and the lactate/pyruvate ratio in any of the control or asphyctic groups

We may thus tentatively conclude that cerebral hypoxia was responsible for the changes observed and that analysis of the CSF lactate/pyruvate ratio may be of clinical importance in cases of neonatal asphyxia Analysis of lumbar CSF will evidently give a somewhat attenuated and delayed picture of cerebral events but may still be useful since repeated analyses should indicate whether restituation is occurring after an incident of asphyxia or if the hypoxia is stationary or if it is increasing in severity The prognostic value of analysis of CSF lactate/pyruvate ratio remains to be confirmed by a long term follow up study of the neonates involved

SUMMARY

The cerebrospinal fluid (CSF) lactate and pyruvate concentrations and the lactate/pyruvate ratios the CSF pH, P_{CO} and bicarbonate concentrations and the simultaneously registered arterial blood pH P_{CO} and P_O were studied in two groups of newborn infants one group of neonates without asphyxia i e a normal control group (19 infants) and a second group of neonates who had sustained perinatal asphyxia (21 infants)

A significant increase of the CSF lactate/pyruvate ratio was observed among the asphyxiated neonates who were studied within 24 hours of the asphyxial incident It is suggested that cerebral hypoxia was responsible for the changes observed, and that analysis of the CSF lactate/pyruvate ratio may be of diagnostic value in perinatal asphyxia

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CPC PRECIPITABLE URONIC ACID CREATININE RATIO IN RANDOM URINE SAMPLES COLLECTED FROM NORMAL CHILDREN

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It is generally thought that estimation of glycosaminoglycan (GAG) excretion for the diagnosis of mucopolysaccharidoses is best performed on a 24 hour collection of urine This is often difficult to obtain in young children and it has been suggested that by relating the amount excreted (as hexuronic acid) to creatinine excretion the problem of incomplete collection can be overcome and a more clear separation of normal from abnormal be obtained (4)

In this hospital laboratory screening for the mucopolysaccharidoses in children seen in the outpatient department has necessitated examining not only incomplete 24-hour urine collections but also many random samples of urine We have therefore found it necessary to determine the normal range for random samples and our results are herein described

MATERIALS AND METHODS

Random samples were collected between 0900 to 1800 hours from 442 normal males and 389 normal females aged 6 months to 19 years who were attending day nurseries or schools in the Bristol Clinical Area. Creatinine was estimated using an autoanalyser method (Technicon method file N 11) GAG were precipitated with cetylpyridinium chloride (CPC) by the method of Di Ferrante (2) using modified volumes to suit the size of the sample available The hexuronic acid content of the precipitated GAG was estimated by the method of Bitter & Muir (1) Results were expressed as mg of hexuronic acid per g creatinine (UA/C ratio)

The same estimations were made on several random samples collected from three normal children during a single 24-hour period and on six consecutive 12 hour collections made between 1200–0000 and 0000–1200 hours from each of two children undergoing investigation of malabsorption

RESULTS

The UA/C ratio showed a skew distribution below the age of five years with the majority of results below the arithmetic mean The upper limit of 95 normal range was calculated from the mean+2 S D of the logarithms of UA/C ratio in these children Above the age of 5 years the upper limit was calculated from the arithmetic values (mean+2 S D) No significant difference could be demonstrated between male and female results in any age group and results for both sexes were pooled in the final analysis No significant change with age was found after the 10th year and results in older children were also pooled for the final analysis The upper limit of normal for each age group and the numbers of samples analysed are shown in Table 1

The results of analysis of several random samples collected from an individual child are shown in Table 2 Although none of the results is outside the normal range for the child s age the highest values were usually found in the first specimen passed in the morn

Table 1 *CPC precipitable uronic acid (UA) per g of creatinine (C) upper limit of 95 % normal range for age*

Age (y)	Number of subjects		UA/C ratio Upper 95 limit Both sexes
	Male	Female	
½–1	20	18	38 4
1–2	13	13	35 6
2–3	18	19	27 2
3–4	13	13	26 9
4–5	13	14	25 7
5–6	10	10	20 7
6–7	15	6	16 5
7–8	12	9	13 6
8–9	17	9	13 6
9–10	16	11	11 3
10–11	19	10	10 3
11–19	273	257	9 1

Table 2 *Random urines collected during a single 24 hour period*

UA = uronic acid C = creatinine

Subject A (male 6½ y)		Subject B (male 9 y)		Subject C (male 8 y)	
Time	UA/C	Time	UA/C	Time	UA/C
0900	15 3	0900	6 7	0830	6 3
1210	11 6	1220	4 7	1400	6 2
1630	7 3	1630	8 6	1800	7 2
1800	8 6	1755	7 5	1930	6 6
1830	10 0	1830	8 8	2000	10 3
1855	12 5	1900	7 5	0800	12 5
0830	9 7	2230	7 8		
		0825	9 9		

ing The results on consecutive 12 hour collections are shown in Table 3 and show highest values consistently in the overnight urine Three samples collected from boys aged 2 3 and 5 years had a creatinine concentration below 10 mg/100 ml and gave a UA/C ratio above the upper limit of normal (44 0, 45 8 and 28 2 respectively) These samples were excluded from statistical analysis

DISCUSSION

Our results for CPC precipitable uronic acid excreted per g of creatinine in random urine samples show the same change with age as 24 hour collections (4) However our results do not show any significant change with age after the 10th year and are slightly lower than the upper 95 % limit (of 14 4 mg per g creatinine) given by Teller et al (4) for 24 hour collection from 12 year olds Our upper limit of normal in children below the age of 5 years is also slightly lower than theirs but inspection of their data shows that all their results are within our normal range suggesting that their calculated 95 % normal range is a little too high in this age group possibly due to the smaller number of samples studied and the statistical weighting effect of four older children who gave higher results than any which we have found If these 4 results are excluded our normal range for random samples encompasses all their results on 24 hour collections We confirm their observation that there is no sex difference in the UA/C ratio

Di Ferrante & Lipscomb (3) have recently underlined the diagnostic limitations of this ratio in random samples since in healthy adults they found wide variations some values exceeding the upper limit of the 24 hour range for the same subject However, inspection of their data shows that as in our results the highest values were obtained in overnight samples their results on random samples collected at other times all falling within the normal 24 hour range for the same subject (Con

Table 3 *CPC AU/C ratio in 12 hour urine collections from two children with coeliac disease*

Sex	Age (y)	Time of collection period in hours					
		0000–1200	1200–0000	0000–1200	1200–0000	0000–1200	1200–0000
M	1³/₁₂	16 0	12 1	12 3	9 3	17 1	16 8
F	1⁶/₁₂	22 4	19 3	26 2	14 5	37 0	29 3

firmed by Di Ferrante personal communication)

It is also apparent that when creatinine concentration is very low (below 10 mg/100 ml) a fall in GAG concentration does not occur to the same extent thus yielding falsely high UA/C ratios

Therefore provided random samples are collected during normal outpatient attendance times and early morning and nocturnal samples and those with very low creatinine content are excluded spurious high results may be avoided and results can be as reliably interpreted as those obtained from a 24-hour collection Since it is frequently impossible to obtain complete 24 hour urine collections from young children this observation is extremely valuable in the detection of mucopolysaccharidoses

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We gratefully acknowl dge the help of the Medical Re earch Committee United Bristol Hospitals for th ir financial assistance Dr D N Challacombe kindly allowed us to u e aliquots of carefully collected 12 hour samples Mrs Sh ila Kitts efficiently arranged the collection of samples from pre school children

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0900	15 3	0900	6 7	0830	6 3
1210	11 6	1220	4 7	1400	6 2
1630	7 3	1630	8 6	1800	7.2
1800	8 6	1755	7 5	1930	6 6
1830	10 0	1830	8 8	2000	10 3
1855	12 5	1900	7 5	0800	12.5
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α FOETOPROTEIN ALBUMIN AND TOTAL PROTEIN IN SERUM FROM PRETERM AND TERM INFANTS AND SMALL FOR GESTATIONAL AGE INFANTS

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It is well known that foetal serum of man and other mammals contains protein fractions not demonstrable or found only in negligible quantities in adult individuals of normal health Using paper electrophoresis Bergstrand & Czar showed (1, 2) that in the human foetus such a protein an α globulin decreases with increasing gestational age The protein now usually called α foetoprotein, was not until recently demonstrable in prematures or in full term newborns However with immunological methods small quantities of this protein have been found in the newborn infant (8 9 10 12 15, 18)

Since the amount of α foetoprotein decreases during gestation to a very low level at term the question arises as to whether the serum level of this protein reflects the degree of foetal maturity If so preterm infants with a weight appropriate for the gestational age should evidence a higher level of α foetoprotein than full term small for gestational age infants with the same weight Preliminary results presented by Lardinois et al (12) and Karlsson et al (10) point in fact in this direction To elucidate this subject further the present study was carried out The purpose was to study α foetoprotein, albumin and total protein in serum from newborn infants of various gestational ages and with various birth weights

MATERIAL AND METHODS

Material

Blood sera were analysed from 165 newborn infants aged 0–1 day and of various gestational ages Gestational age in weeks was calculated from the first day of the mother s last menstrual period (e g 40th week days 274–280) in accordance with the principles for constructing the Swedish standard curves for the relation between birth weight and gestational age (4 19) Blood samples were collected as described by Ekelund et al (3)

Preterm was defined as a gestational age less than 39 weeks term 39–42 weeks and postterm more than 42 weeks

Appropriate for gestational age infants (AGA) had birth weights within normal limits (10th–90th per centile) for the gestational age

Small for gestational age infants (SGA) had birth weights below normal for gestational age

Large for gestational age infants (LGA) had birth weights above normal for gestational age

The distribution of birthweights in relation to gestational age is illustrated in Fig 1

Four main groups of infants were considered I) preterm AGA (25–38 weeks) with the subgroups *a*) preterm AGA 25–32 weeks and *b*) preterm AGA 33–38 weeks II) preterm SGA (33–38 weeks) III) term AGA and IV) term SGA The Swedish standard curves do not cover age ranges below 33 weeks but as could be judged from a comparable standard curve (21) the infants below 33 weeks were all AGA and were consequently classified as such The values from the preterm LGA infants were included in the

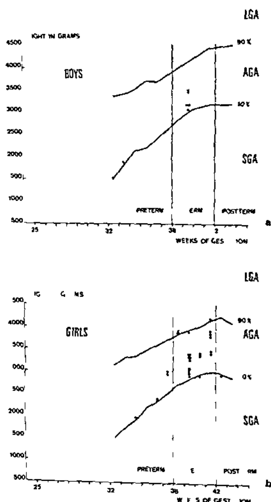


Fig 1 Classification of the material according to birth weight, gestational age and sex. Swedish standard curves for intrauterine growth (19)

preterm AGA group The term LGA, the postterm LGA and postterm AGA infants were included in the term AGA group This was done because there were few individuals in these groups and the values for α foetoprotein, albumin and total protein did not differ significantly from those in the AGA groups to which they were referred

α Foetoprotein and albumin were determined by electrophoresis in agarose gel containing antibodies according to Laurell (13) using specific antisera produced in rabbits as described in detail previously (10)

Total protein was determined according to Lowry et al. (14) using ribonuclease (AICC Chicago lot no DCO 850) as a reference

Statistical methods

Conventional statistical methods were used for calculating the ... ns standard deviations (SD) and standard errors of the means (SEM) Significance of differences between means was analysed by the Student s *t* test.

RESULTS

In sera from preterm AGA newborns the α foetoprotein arc usually was visible in immunoelectrophoretic tests with unabsorbed antifoetal sera In the SGA infants and the term AGA group the arc was essentially absent except in sera from a few preterm SGA infants However with the use of antifoetal sera absorbed with adult serum the α foetoprotein arc was usually visible also in the term AGA group

The birthweights and the levels of α foetoprotein albumin and total protein for preterm AGA term SGA and term AGA infants are recorded in Fig 2 and Table 1 The α foetoprotein levels were numerically higher for boys than for girls This difference was most pronounced in the term AGA group but was not significant No similar tendency was noted for the albumin and total protein levels

The α foetoprotein levels were significantly higher in preterm AGA infants than in term AGA and term SGA infants (Table 2) However there was no significant difference be

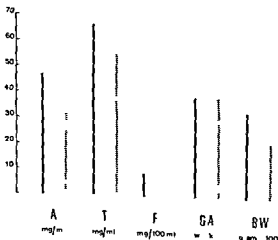


Fig 2 Mean albumin (*A*) total protein (*T*) levels α foetoprotein (*F*) gestational ages (GA) and birth weights (BW) for the three groups term AGA (—) preterm AGA (---) and term SGA ( ) Cf the values given in Table 1

Table 1 *Mean levels (± SEM) of α foetoprotein (F), albumin (A) and total protein (T) in serum of newborns of various gestational ages (GA) and birth weights (BW)*
n = number of individuals

Group		F (mg/ml)	A (mg/ml)	T (mg/ml)	BW (kg)	GA (weeks)	n
I (*a*+*b*)	Preterm AGA (25–38 w)	0 31 ±0 03	30 16 ±1 25	44 59 ±1 80	2 33 ±0 11	34 5 ±0 5	51
(*a*)	Preterm AGA (25–32 w)	0 57 ±0 06	26 2 ±1 7	38 8 ±4 1	1 39 ±0 10	29 9 ±0 5	19
(*b*)	Preterm AGA (33–38 w)	0 20 ±0 02	32 70 ±1 39	46 96 ±1 83	2 72 ±0 11	36 4 ±0 3	32
II	Preterm SGA (33–38 w)	0 26 ±0 08	30 3 ±2 4	43 8 ±1 6	2 15 ±0 05	36 9 ±0 4	6
III	Term AGA (39–42 w)	0 08 ±0 01	47 81 ±1 37	68 20 ±1 48	3 47 ±0 37	40 6 ±0 1	89
IV	Term SGA (39–42 w)	0 09 ±0 02	37 87 ±2 43	54 48 ±2 96	2 37 ±0 09	40 6 ±0 2	19

tween term SGA and term AGA infants The levels in the preterm SGA infants were the same as those in the preterm AGA infants (Table 1)

Preterm AGA infants with low gestational age (25–32 weeks) showed a significantly higher level ($p < 0\ 001$) of α foetoprotein than preterm AGA infants with higher gestational age (33–38 weeks)

The serum albumin levels of term AGA infants were significantly higher than those of term SGA infants (Table 2) This was true also for the total protein levels The albumin and total protein levels were significantly lower in preterm AGA infants than in those of the term AGA group No differences were found between infants in the preterm AGA and preterm SGA groups

DISCUSSION

The results of the present investigation showed that the levels of α foetoprotein differed significantly between preterm AGA and term

Table 2 *Significance levels for α foetoprotein albumin and total protein between the groups from Table 1*

	Preterm AGA	Preterm SGA	Term AGA	Term SGA
α Foetoprotein				
I preterm AGA	—	—	***	***
II preterm SGA		—	***	***
III term AGA			—	—
IV term SGA				—
Albumin and total protein				
I preterm AGA	—	—	***	***
II preterm SGA		—	***	*
III term AGA			—	***
IV term SGA				—

* $p < 0\ 05$ ** $p < 0\ 01$ *** $p < 0\ 001$

AGA infants The same was true for preterm SGA and term SGA infants However there were no significant differences between term AGA and term SGA or between preterm AGA and preterm SGA infants

A significant difference existed between preterm AGA and term AGA infants in albumin levels but not between preterm SGA and term SGA On the other hand the albumin levels significantly differed between term AGA and term SGA infants but not between preterm AGA and preterm SGA infants The trends found for albumin were also found for the total protein levels This could be expected since albumin is the predominant protein in serum

It is evident that the α foetoprotein is a good indicator of gestational age This conclusion can be drawn from the fact that the α foetoprotein levels did not differ significantly between groups of infants of the same gestational age but with various birth weights whereas the levels differed between groups of infants of various gestational ages but with the same birth weight

Our observations on α foetoprotein are in accordance with the preliminary results of Lardinois et al (12) who found that the concentrations were higher in "premature than in full term infants However their statement that small for date infants had low or negligible amounts as compared with full term babies is at variance with our findings A reason for these different results may be that the methods used by Lardinois et al —immuno electrophoresis and polyacrylamide gel electrophoresis—only gave a semiquantitative measure of the α foetoprotein levels

It has earlier been reported that the total protein levels of premature infants are lower than those for full term infants (16 17 20) These differences are dependent on the albumin l vels The present investigation confirms these findings

The most prominent characteristic for SGA and AGA infants was the significant difference between albumin and total protein levels in term AGA and term SGA infants Since the liver is known to be the site of synthesis of the serum albumin it may be questioned whether the differences registered between term AGA and term SGA infants reflect a lower synthesis or higher catabolic rate of albumin in the liver of the SGA infants (11) Saito et al (17) studied the plasma protein pattern in infants of varying birth weights and found lower albumin levels and thus lower total protein levels in the lower weight groups Unfortunately the report did not give a clear picture of the gestational age of the infants and its influence on albumin and total protein levels

Our conclusion that the α foetoprotein level is a good indicator of gestational age is supported by previous determinations using the same material as in the present investigation The correlation coefficient for the linear regression between α foetoprotein and gestational age was higher than that between birth weight and gestational age (10) Neither albumin nor total protein showed such high correlations with gestational age

It is well documented that birth weight is an unreliable index of gestational age (7) Recently Finnstrom has shown that a number of parameters can be used singly or in combinations to increase the reliability of estimating gestational age (5 6 7) Using his equations for calculating the 95 confidence limits we found the value $\pm 4\ 3$ weeks for α foetoprotein $\pm 6\ 2$ weeks for albumin and $\pm 6\ 1$ weeks for total protein The corresponding value for birth weight in our material was $\pm 4\ 9$ weeks as compared with $\pm 28\ 9$ days in Finnstrom s studies The lowest value of 95 confidence limit obtained by Finnstrom for a single parameter (external characteristics) was ± 24 days and for a combination of five different parameters $\pm 19\ 5$ days

It may be concluded that determination of α foetoprotein can be added to the procedures used for estimating gestational age By use of a material more precisely defined than the present the confidence limits may even be nar

rowed For practical purposes, however, the method has certain disadvantages, e g, serum samples should preferably be collected within the first 2–4 hours after birth

SUMMARY

α Foetoprotein, albumin and total protein were analysed in serum from infants of various gestational ages and with various birth weights Assays were performed with electro phoresis in agarose gel containing specific anti bodies

The α foetoprotein level was significantly higher in preterm than in term infants both with birth weights appropriate for gestational age No difference was noted between infants born at term with birth weights appropriate and low for gestational age

The albumin and total protein levels were significantly lower in term infants small for gestational age than in term infants appro priate for gestational age

The results of this study show that the serum levels of α foetoprotein may be used as an indicator of gestational age

ACKNOWLEDGEMENTS

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POSTNATAL CHANGES OF ALPHA FOETOPROTEIN ALBUMIN AND TOTAL PROTEIN IN HUMAN SERUM

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It is well established that one of the characteristics of foetal serum of man and other mammals is the presence of a special protein fraction α foetoprotein (2 4 6 7 8 11 13 14 21 23 24 26) Little is known about the physiological role of this protein and the factors regulating its synthesis and catabolism Whether the synthesis and function of α foetoprotein in any way is connected with or has any influence upon the growth and development of the foetus is unknown

In certain species neonates appear to continue the synthesis of α foetoprotein It was recently shown that the α foetoprotein levels in the serum of 1 week old piglets were significantly higher than those at birth After 1 week of age the levels began to decline (11)

A detailed investigation of the postnatal changes of α foetoprotein may give further information about the factors responsible for the synthesis and catabolism of this protein

This study concerns α foetoprotein albumin and total protein in preterm and full term newborns at birth and during the first months of life

MATERIAL AND METHODS

The total material consisted of 330 infants with a gestational age of 25 44 weeks One subgroup consisting of 171 newborns aged 0–1 day was used to study the levels of α foetoprotein albumin and total protein as related to gestational age The second subgroup included 159 infants from whom one blood sample was collected at times varying from 5 min to 6 months after birth the purpose of the second group was to study postnatal changes of α foetoprotein albumin and total protein.

The blood samples were collected as described by Ekelund et al (5) In all newborns aged 0–5 days blood was drawn by a catheter in one of the umbilical vessels Blood from older infants was obtained by venipuncture After centrifugation the serum samples were stored at −20 C for at most 24 months befo e analysis

Antisera to serum proteins from human foetuses (17–20 weeks old) and newborn were produced in rabbits (2 5–3 5 kg) Each rabbit received two injections of the antigen with an interval of 5–6 weeks The antigen solution was prepared as follows 1–2 ml of foetal serum or 2–4 ml of neonatal serum were diluted with 0 15 M NaCl solution to a final volume of 5 ml Each of the antigen solutions was mixed with 5 ml Freund Bacto Adjuvant Incomplete (Difco Laboratories Detroit) and separately emulsified with a Sorwall homogenizer at low speed At both injections 1–2 ml of the antigen/adjuvant mixture was administered intramuscularly and 6–8 ml were administered under the scapulae One week after the second injection the immunized rabbits were bled (3–4 times every s cond day) by heart puncture The antisera to foetal serum thus obtained were rendered specific to α foetoprotein by absorption with human sera from healthy adult blood donors in the volumic proportions 1 0 1–1 0 8 at 37 C for 4–5 hours The effect of the absorption was controlled by *immunoelectrophoresis*

No α foetoprotein *precipitates* were detected by immunoelectrophoresis when antisera to neonatal era were absorbed with serum from adult individuals and then tested with neonatal and foetal serum samples

Human α foetoprotein (albumin contaminated) was obtained after block electrophoresis (pH 8 6 Veronal buffer) of pooled foetal sera in pevikon (Pevikon

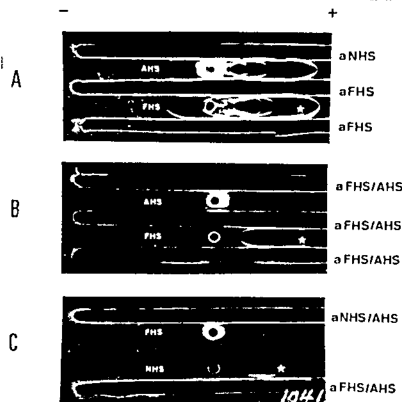


Fig 1 Immunoelectrophoretic demonstration of α foetoprotein (×) (*A*) Blood serum from adult human (*AHS*) and a foetus (*FHS*) tested with antifoetal serum (*aFHS*) and antineonatal serum (*aNHS*) prepared in rabbits (*B*) AHS and FHS tested with aFHS absorbed with AHS (*aFHS/AHS*) (*C*) Foetal and neonatal human sera tested with antisera aNHS and aFHS absorbed with AHS (*aNHS/AHS aFHS/AHS*)

C 870 Stockholms Superfosfat AB) This preparation procedure is rather difficult with human foetal sera as the albumin which is present in high amounts migrates only a little faster than the α foetoprotein However by cutting out fractions just behind the albumin and rerunning these fractions rather pure preparations of α foetoprotein were obtained Starting with 3 ml of foetal serum final preparations were obtained which contained α foetoprotein and albumin in the proportions 3 1 The albumin level was determined by electrophoresis in agarose gels containing antibodies as described below The content of α foetoprotein in the samples was estimated by subtracting the albumin from the total protein content of the preparation as determined according to Lowry et al (17) These preparations were used as references for determination of α foetoprotein levels

The α foetoprotein levels were determined by electrophoresis in agarose gels containing antibodies according to the method described by Laurell (16) Runs were usually performed at 2–3 V/cm for 12–18 hours at +5 C and included 3–6 various serial dilutions of samples of known α foetoprotein content The buffered 1 5 agarose gel (l Industrie Biologique Françai e S A mixed with Difco Special Agar Noble in the proportions 20 1) contained absorbed antifoetal serum usually in the proportions 30 2 5 Samples to be analysed were either undiluted or diluted 11×–121× in order to give precipitates not longer than 50 mm The concentration of α foetoprotein in the original serum sample was easily calculated by measuring the height of the peaks formed and comparing them with those formed by the solutions of known concentrations and then multiplying by the dilution factor

The absorption of antisera was controlled by immunoelectrophoresis on sera from various stages of foetal development in order to avoid influence of increasing concentrations of other proteins

The albumin content was determined by electrophoresis in agarose gel containing antiserum to human neonatal serum (Agarose/antiserum = 15/0 7–0 1) Serial dilutions of Standard Human Serum (Behringwerke AG) were used as reference The samples to be analysed were diluted 121× and the same electrophoretic procedure as described for the α foetoproteins was used (heights of the precipitates 20–36 mm)

Total protein was determined with the Folin-Ciocalteau reagent according to Lowry et al (17) Ribonuclease (AICC Chicago lot no DCO 850) or Bovine serum albumin (Fraction V Sigma) were used as standards

Statistical methods Conventional statistical methods were applied for calculating arithmetic mean standard deviation and standard error of the mean (S E M) α Foetoprotein albumin and total protein were each correlated with gestational age with birth weight and with each other using linear regression analysis

In order to study whether the regression of α foetoprotein level albumin level and total protein level on gestational age was nonlinear second and third degree polynomial regression was used To determine whether the analyses were influenced by unstable variance the square roots and log values of the various parameters were also analysed The same results were obtained in each case

RESULTS

A precipitation arc corresponding to α foetoprotein was always observed when foetal sera were tested with antifoetal serum (Fig 1 *A*) This arc could not be distinguished from other precipitates when neonatal and adult human sera were tested with antisera to foetal neonatal and adult blood sera Using antifoetal serum absorbed with adult serum the specific α foetoprotein arc could be demonstrated in foetal sera (Fig 1 *B*) but also in neonatal sera although the precipitation line was very faint and for some sera not visible (Fig 1 *C*) In the absorption tests one additional line was registered in the β globulin region of the immunoplates This line disappeared when the antiserum specific to α foetoprotein was absorbed with human foetal haemoglobin This did not interfere with the interpretation of the results

The α foetoprotein levels were high in newborns with gestational ages of 25–30 weeks but decreased successively with increasing gestational age (Fig 2) The mean values at term

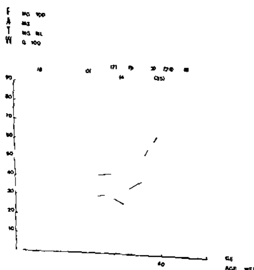


Fig 2 Birth weights (gram × 100 Δ—Δ) and levels of α foetoprotein (mg/100 ml ●—●) albumin (mg/ml ○—○) and total protein (mg/ml □—□) of newborn infants of the gestational ages 25–43 weeks Figures within parentheses indicate number of individuals studied Note that the various parameters are expressed in different units of magnitude

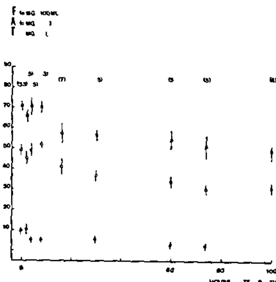


Fig 3 Mean levels (±S E M) of α foetoprotein (mg/100 ml ●—●) albumin (mg/ml, ○—○) and total protein (mg/ml □—□) in newborn infants during the first 100 hours after birth Figures within parentheses indicate number of individuals studied

were approximately eight times lower than those registered at the 25th–30th weeks of gestation The albumin and total protein levels increased with increasing gestational age The mean values for newborns at term were approximately twice those at the 25th–30th weeks of gestation In sera from newborns delivered after 43 weeks of gestation the albumin and total protein levels were slightly lower than those from newborns delivered at term while the α foetoprotein levels were the same in both groups

The postnatal changes of the levels of α foetoprotein albumin and total protein are recorded in Fig 3 During the first 20 hours after birth the mean levels of α foetoprotein decreased from 0 10 to 0 02 mg/ml During the 2nd–4th days after birth the levels were rather constant but then decreased and were 0 006 mg/ml 2 weeks after birth Traces of α foetoprotein were recorded in only a few sera collected 7 weeks and 5 months after birth The mean albumin levels decreased from 50 to 30 mg per ml and the total protein from 70 to 50 mg per ml within the first 5 days

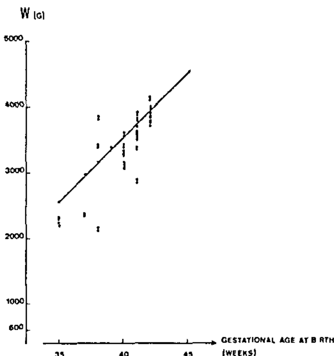


Fig 4 Correlation between gestational age (33–42 weeks) and birth weight The regression line is indicated in the figure and can be derived from the formula $Y = -2\ 79 + 0\ 16x$

after birth (Fig 3) These levels were recorded also 40–150 days *post partum*

Great individual variations were observed in the postnatal development of all the protein fractions considered The variations were most pronounced for α foetoprotein In some cases the levels decreased within the first days after birth, while in others they were main tained approximately constant In still other cases the levels slightly increased

The correlation between birth weight and gestational age and between the α foetoprotein level and gestational age is shown in Figs 4 and 5 These figures comprise data from in fants of 33–42 weeks of gestational age The coefficient of correlation between α foeto protein and gestational age was higher thar that between birth weight and gestational age Similar calculations for albumin and total pro tein levels in relation to gestational age als revealed lower correlation coefficients Thes calculations were extended to include th whole material (25–42 weeks of gestation) Th coefficients for the correlations in all possibl combinations between α foetoprotein albumi and total protein levels gestational age an birth weight are given in Table 1

The results show that the correlations be tween the parameters considered in Table are approximately linear over the whole peric (25–42 weeks of gestation) The highest co efficients of correlation were found betwee albumin and total protein (+0 87) and b tween α foetoprotein and gestational a (−0 82) All correlations given in Table were significant ($p < 0\ 001$)

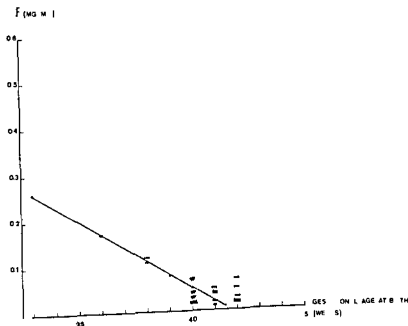


Fig 5 Correlation between gestational age (33–42 weeks) and α foetoprotein level (mg/ml) The regression line is indicated in the figure and can be derived from the formula $Y = 1\ 25 - 0\ 03x$

After elimination of the influence of gestational age the partial coefficients of correlation were calculated for α foetoprotein level with birth weight albumin level with birth weight and total protein level with birth weight None of these partial coefficients of correlation were significant

The *α foetoprotein levels in boys were* usually higher than those in girls but the difference between the means was not significant

Table 1 *Significant coefficients of correlation for the possible combinations of the parameters α foetoprotein (F) albumin (A) and total protein (T) levels birth weights (BW) and gestational ages (GA)*

	GA	BW	F	A	T
GA	1 00	0 77	−0 82	0 59	0 60
BW	—	1 00	−0 65	0 51	0 50
F	—	—	1 00	−0 41	−0 47
A	—	—	—	1 00	0 87
T	—	—	—	—	1 00

DISCUSSION

The concentrations of α foetoprotein albumin and total protein registered in the present investigation were principally in agreement with those *given in studies* where other methods have been used (2 6 8 20) The values of *α fo* toprotein must not be considered as definite because available methods of isolation *do not allow* complete separation from albumin (8) The method of Laurell (16) *seems to have some advantages over other quantitative* immunodiffusion methods as was also pointed out by Hirsch Marie & Conte (9)

The α foetoprotein levels showed great individual variation for each particular gestational age This variation included both the values at birth and the disappearance rate from serum after birth

In previous studies on postnatal changes of α foetoprotein of man and other mammals it has been claimed that the synthesis ceases im*mediately aft r birth* resulting in successively diminishing amounts during the neonatal period With the Laurell *method it* was possible to follow these changes more in detail Recently it was shown for newborn piglets that the α foetoprotein level increased in serum during the first week of life *in spite of the* fact that the blood volume increased *during* this period (11) In the present investigation *no such* increase could be noted for α foetoprotein of newborn infants The results are in accordance *with those* published by Gitlin & Boesman (8) but in their studies the changes of *α* foetoprotein were followed at 2–3 day intervals Consequently the changes occurring during the first hours after birth could not be registered

In the present material there was a significant drop in the serum concentrations of α foetoprotein within the first 10 *hours* after birth Because of the heterogeneity of the *material* concerning e g gestational age time of cord clamping (27) and time of the first feeding the exact pattern of this decrease can not be given in detail However *it is* likely that a real decrease of α foetoprotein took *place within the first 4–5* hours after birth Results from analysis of consecutive *serum samples obtained from the* same individual support *this* conclusion A similar reduction but occurring at 10–20 hours after birth was noted for albumin and total protein During the following 2–4 days these levels continued to decrease significantly whereas the level of α foetoprotein was rather constant

The interpretation of these results is difficult The fact that the α foetoprotein level did not vary significantly over a period of 10–100 hours after birth may depend on a continued low rate of synthesis It must be stressed that with the methods used evidence for such a synthesis *is* only indirect The results obtained by Seppala et al (24) that the α foetoprotein level *remained rather high after* multiple blood transfusions may support the suggestion that a postnatal synthesis of *α foetoprotein* can occur

The decreasing levels of albumin and total protein after birth are in accordance with pre

viously published results (10) but the decrease found in the present study was more pronounced The explanation for this is not clear In this study the antiserum used for quantitation was prepared by use of neonatal serum as an antigen and with this method it is possible that the decrease in albumin and total protein level caused by catabolism of a postulated foetal albumin is better reflected (15, 19) Further studies on serum albumin in foetuses and neonates are needed to clarify this point

It is an important and still unanswered question in developmental biology whether any of the serum proteins have an influence upon or in any way are connected with the growth of the foetus Such a quality could possibly be ascribed to α foetoprotein since it has been shown that foetal proteins can promote in vitro growth of cell cultures (18 22, 28) These experiments have been performed with fetuin a foetal protein from calf serum (21) However this protein is probably not homologous to the α foetoprotein of man, as has earlier been claimed (1, 12) In the present study it is true that the coefficients of correlation were significant between birth weight and each of the other three parameters α foetoprotein, albumin and total protein, but judging from the calculations of partial coefficients of correlation these three parameters and birth weight are all dependent on gestational age From this it may be concluded that even if the α foetoprotein level is, for example high for a given gestational age this does not mean that the birth weight should be high Further support for this conclusion was obtained in a study including small for gestational age infants (3) where no differences in α foetoprotein levels were observed between infants of the same gestational ages but with abnormally low or high birth weights

The fact that the α foetoprotein level is correlated with gestational age may offer a new possibility for the estimation of maturity of newborn infants This finding will be discussed elsewhere (3)

SUMMARY

α Foetoprotein albumin and total protein was assayed in serum of newborn infants with electrophoresis in agarose gels containing antibodies and with the Lowry method

Within 4–6 hours of birth the α foetoprotein level decreased A similar drop was registered for albumin and total protein 10–20 hours after birth During the following 2–4 days after birth the levels of albumin and total protein continued to decrease whereas the α foetoprotein level was rather constant These results were tentatively interpreted as a continued though low, synthesis of α foetoprotein after birth

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viously published results (10) but the decrease found in the present study was more pronounced The explanation for this is not clear In this study the antiserum used for quantitation was prepared by use of neonatal serum as an antigen and with this method it is possible that the decrease in albumin and total protein level caused by catabolism of a postulated foetal albumin is better reflected (15, 19) Further studies on serum albumin in foetuses and neonates are needed to clarify this point

It is an important and still unanswered question in developmental biology whether any of the serum proteins have an influence upon or in any way are connected with the growth of the foetus Such a quality could possibly be ascribed to α foetoprotein since it has been shown that foetal proteins can promote in vitro growth of cell cultures (18, 22 28) These experiments have been performed with fetuin a foetal protein from calf serum (21) However, this protein is probably not homologous to the α foetoprotein of man as has earlier been claimed (1, 12) In the present study it is true that the coefficients of correlation were significant between birth weight and each of the other three parameters α foetoprotein albumin and total protein but judging from the calculations of partial coefficients of correlation these three parameters and birth weight are all dependent on gestational age From this it may be concluded that even if the α foetoprotein level is for example high for a given gestational age this does not mean that the birth weight should be high Further support for this conclusion was obtained in a study including small for gestational age infants (3) where no differences in α foetoprotein levels were observed between infants of the same gestational ages but with abnormally low or high birth weights

The fact that the α foetoprotein level is correlated with gestational age may offer a new possibility for the estimation of maturity of newborn infants This finding will be discussed elsewhere (3)

SUMMARY

α Foetoprotein albumin and total protein was assayed in serum of newborn infants with electrophoresis in agarose gels containing antibodies and with the Lowry method

Within 4–6 hours of birth the α foetoprotein level decreased A similar drop was registered for albumin and total protein 10–70 hours after birth During the following 2–4 days after birth the levels of albumin and total protein continued to decrease whereas the α foetoprotein level was rather constant These results were tentatively interpreted as a continued, though low, synthesis of α foetoprotein after birth

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THE IN VITRO UPTAKE OF LYSINE AND ALANINE BY HUMAN JEJUNAL MUCOSA IN PROTEIN CALORIE MALNUTRITION, IN GASTROENTERITIS AND AFTER NEOMYCIN

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A Technical Report of the World Health Organisation (28) stated that Studies are needed on the absorption and excretion of other substances in the gastro intestinal tract including protein and fat, especially in enteric bacterial and helminthic infections The occurrence of malabsorption in the small intestine in some diseases is a factor that might well influence the malnourished host, in this respect the physiological consequences of giardiasis need consideration

This study of the capacity for absorption of two aminoacids by the mucosa of the small bowel of sick children used an in vitro technique A specimen of living epithelium was obtained by peroral biopsy from the region of the duodeno jejunal flexure (3) and was incubated in isotonic solution containing radioactively labelled lysine and alanine The concentration in the tissue after a steady state had been reached was compared with that in the surrounding medium and expressed as the distribution ratio (D R) which was a measure of the absorptive power of the mucosa (17, 26) A larger ratio reflected more uptake

MATERIAL AND METHODS

The twelve patients studied fell into two groups Five children (age range 1 year 5 months to 4 years) were admitted to hospital for treatment of the clinical syndrome of kwashiorkor apathy characteristic changes in skin and hair oedema and nutritional hypoalbuminaemia (less than 2 g/100 ml) Seven children (age range 1 to 3 years) were selected as requiring intravenous therapy for acute gastroenteritis They were typical of the many malnourished children who are prone to repeated attacks of diarrhoea Only one was above the third Boston percentile for weight and in only two was the serum albumin above 4 g/100 ml Salmonella were grown from two cases and no bacterial pathogens from the others

Fresh unstained duodenal juice was examined under the microscope for the trophozoites of Giardia lamblia They were found in four children in each group

Lactose intolerance was diagnosed clinically if large fluid acid stools became macroscopically normal when on a lactose free formula of casilan (4) It occurred in two of the first group and three of the second

The subjects were given glucose and electrolyte solutions on the day of admission and usually biopsy was taken the next morning In those with kwashiorkor biopsy was repeated the next day and the whole estimation duplicated Treatment was with a standard regime of increasing diet with vitamin and mineral supplements antibiotics if indicated and mepacrine if Giardia had been found In three children biopsy was done again during recovery In the group with gastroenteritis tissue was obtained before treatment was begun Then milk was introduced and neomycin administered in a dose of 10 mg/kg body weight eight times a day until the second biopsy three or four days later No other drugs were given

PROCEDURE

Estimation of uptake was performed separately on each specimen The method and calculation of results was based on that of Rosenberg et al (22) One piece of mucosa was obtained at a time the weight of which varied between 2 and 8 mg—less than commonly

Table 1 *Results in Kwashiorkor*

Patient	Duration of treatment (d)	Lysine D R	Lysine Mean	Lysine S D	Alanine D R	Alanine Mean	Alanine S D
S N	1	7 4	7 4		6 7	7 0	
	2	7 0			7 4		
	14	14 5			4 2		
	22	6 1	5 3		8 8	6 7	
	60	4 7			8 0		
	62	5 0			6 6		
M M	1	2 5	3 5		6 9	9 2	
	2	4 5			11 4		
	15	4 9			7 2		
	16	4 6	5 4		8 5	9.2	
	21	7 7			12 1		
	25	4 5			9 0		
A D	0[a]	8 2	6 4		*11 1*	9 0	
	1	4 6			7 0		
	15	2 6			3 2		
	17	6 7	6 3		6 9	6 6	
	24	6 7			7 1		
	58	9 0			9 3		
D M	0	4 1	5 4		6 6	8 7	
	0	6 8			*10 8*		
E H	10	9 0	9 45		7 6	8 7	
	11	9 9			9 9		
Total for group	0–2		5 6	1 92		8 5	2 14
	14–62		6 6	1 70 $p>0\,5$		7 8	2 17 $p>0\,5$

[a] See text

available in animal experiments This prevented performin estimations in parallel and submitting it to other investigations such as histology or determination of inulin space

Immediately after biopsy it was placed in Krebs-Ringer s solution at 37 C gassed with 95 oxygen and 5 % carbon dioxide It was kept in a shaking metabolic incubator for one hour with both L lysine labelled with *Tritium* at a concentration of 0 33 Mol/l and L alanine labelled with C at 2 5 μMol/l These were chosen as being important aminoacids with separate *transport mechanisms* The choice of their concentrations was arbitrary but was the *same* as we had used before The tissue was then rinsed blotted weighed and boiled in water for five minutes It was re weighed after baking dry and the total wat r content obtained by *subtraction* Intra-cellular fluid volume was assumed to be 80% of this and *extra-cellular* volume 20% The radioactivity in an aliquot of the boiled *water* and of the incubating medium was counted using a dioxane counting fluid and two channels of a scintillation counter (Beckman LS 200) *From this* the concentrations and so the D R could be derived

To serve as inert controls aerobic metabolism was inactivated *in one test* by incubation under nitrogen instead of O CO and in another by dinitrophenol at a concentration of 10^{-4} M (10)

RESULTS

The individual distribution ratios found in the patients with kwashiorkor are listed in Table 1 There was no significant difference between the uptakes found at the start of treatment and later in its course

Agreement between duplicated readings was probably satisfactory for a biological experiment of this type but two particular comments should be made In the case of S N the third ratio for lysine was an isolated high one and for alanine rather low probably it should be disregarded In A D the first ratios for both acids are high and the third low but neither were confirmed by the repeat

Table 2 shows the results in acute gastroenteritis and after treatment with neomycin There was no significant difference between the two groups of values The D R s were lowest in B F and C K where the intervals between administration and biopsy were the

Table 2 *Results in gastroenteritis with neomycin*

Patient	Duration of treatment (d)	Interval between test dose of drug & biopsy (h)	D R Lysine		D R Alanine
M S H	0	—	6 2		10 4
	0	—	3 7		7 7
	4	16	5 5		11 5
B F	0	—	6 0		11 2
	4	¾[a]	2 7		5 6
P V D	0	—	3 0		6 5
	3	4	3 8		7 8
N D	0	—	3 3		7 1
	4	3	6 5		9 5
S S	0	—	2 9		7 5
	3	4½	3 6		5 4
D S	0	—	4 4		11 5
	3	5½	5 7		16 0
C K	3	¾[a]	2 6		4 8
Total of group	0	Mean 4 2	S D 1 38	Mean 8 8	S D 2 14
	3 4	4 3	1 52	8 6	4 09
			$p > 0 5$		$p > 0 5$

[a] See text

shortest However even if uptake were reduced it was for too short a time to be a practical disadvantage

Although the ratios for alanine were similar the ratios for lysine were somewhat lower in Table 2 than they were in Table 1 This may not represent an actual difference in function in the two conditions but be explained by a mechanical failure of the counter which resulted in a delay and resetting of voltage before the samples from the gastroenteritis group were counted

The ratios obtained for the inactivated samples are shown in Table 3 Although they were much lower than the others they were over unity so some uptake had occurred It is presumed that this was caused by adsorption on to the surface of the specimen in addition to passive diffusion into the tissue fluid

Table 3 *Results in controls*

Patient		D R for Lysine	D R for Alanine
M B	Nitrogen used instead of O_2 CO_2 mixture	0 4	1 3
B F	In the presence of 2–4 dinitrophenol	1 8	2 2

DISCUSSION

Uptake is only one aspect of transport across the intestinal wall but it is an active process for aminoacids as is confirmed here by the difference between the results in the physiological and poisoned conditions Occasionally substances may be incorporated into larger molecules without being passed on—leucine fed to rats starved of protein was found particularly within the cells of the jejunum (11) —but this is unusual

Evaluation of the values for D R must be relative as normal children were not studied Here comparison can be made between the ratios before and after treatment in the same patient and within the group The results were of the same order as those of McCarthy et al

in similar work with adult jejunal epithelium who found the D R for lysine to be 5 7 (18) and of our own in children with nutritional rickets

Investigation of the function of the mucosa is relevant in each of the conditions exemplified here In kwashiorkor there is diarrhoea (27) histological changes in the jejunal epithelium (3 6 25) and impaired activity of disaccharidases (4) Although digestion of protein in the lumen of the gut may not proceed as far as to aminoacids their defective absorption might enhance the effects of low dietary intake On the other hand absorption can be increased (7 15) Aminoacids particularly alanine (1) in the blood quickly rose when milk was introduced to children with kwashiorkor and overflowed in the urine (23) In observations using methods similar to these gut slices from rats on a diet containing no protein took up more lysine and glycine than those from animals on a 5% protein diet (which had produced a kwashiorkor like syndrome) or from controls on a full diet (16) In the determinations reported here however neither depression nor enhancement of mucosal absorptive activity was demonstrated in the actual human situation

Bacteria and mucosa interact germ free animals have more regular villi wider brush border and better absorption of xylose than normal (14) while in tropical sprue normal villi are decreased and micro-organisms increased (8) Repeated attacks of acute gastroenteritis occur in association with malnutrition and their effects may summate with it (24 28) Malabsorption of sugars may be found Absorption of aminoacids is important for although oral intake of protein may be low endogenous loss into the bowel may be considerable in secretions exudate blood and sloughed cells (19) However our findings do not confirm the expectation of inadequate uptake in gastroenteritis

The effect of neomycin is of importance because although a known cause of malabsorption (2 9 20) and histological change (12) it is much used in the treatment of gastroenteritis In this study neomycin was given in standard therapeutic dose to patients who were vulnerable in that they were malnourished and had intestinal disease Though the ratios were rather variable it was not shown to inhibit absorption

Giardia lamblia live in the mucus of the small intestine in close association with the mucosa which they may invade (5) It is often found in malnourished children (3) and was seen in eight of our cases The protozoan may cause acute diarrhoea or chronic malabsorption (12 21) but it may be asymptomatic and its usual clinical significance is uncertain The present of giardia was not associated with low D R s and no direct effect on the mucosa has been demonstrated here

Intolerance of milk caused by low lactase activity in the epithelium was found in five members of this series Absorption may not be affected of substances other than lactose water and electrolytes cases of kwashiorkor with intolerance can be cured with milk alone These results were not at variance with this clinical observation and did not show impairment of aminoacid uptake in the children who had large acid stools while on milk

Repetition of these studies with material from more seriously ill patients in different experimental conditions or with other aminoacids might produce other results but no evidence has been found that there was a change in aminoacid handling in these diseases

SUMMARY

The uptake in vitro of lysine and alanine by jejunal mucosa was investigated in kwashiorkor acute gastroenteritis and after neomycin therapy Some of the children also had giardiasis and lactose intolerance No consistent evidence of abnormality was found in any of these diseases under the conditions of the study

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shortest However even if uptake were reduced it was for too short a time to be a practical disadvantage

Although the ratios for alanine were similar, the ratios for lysine were somewhat lower in Table 2 than they were in Table 1 This may not represent an actual difference in function in the two conditions but be explained by a mechanical failure of the counter which resulted in a delay and resetting of voltage before the samples from the gastroenteritis group were counted

The ratios obtained for the inactivated samples are shown in Table 3 Although they were much lower than the others, they were over unity, so some uptake had occurred It is presumed that this was caused by adsorption on to the surface of the specimen in addition to passive diffusion into the tissue fluid

Table 3 *Results in controls*

Patient		D R for	
		Lysine	Alanine
M B	Nitrogen used instead of O_2 CO_2 mixture	0 4	1 3
B F	In the presence of 2-4 dinitrophenol	1 8	2 2

DISCUSSION

Uptake is only one aspect of transport across the intestinal wall but it is an active process for aminoacids as is confirmed here by the difference between the results in the physiological and poisoned conditions Occasionally substances may be incorporated into larger molecules without being passed on—leucine fed to rats starved of protein was found particularly within the cells of the jejunum (11) —but this is unusual

Evaluation of the values for D R must be relative as normal children were not studied Here comparison can be made between the ratios before and after treatment in the same patient and within the group The results were of the same order

in similar work with adult jejunal epithelium who found the D R. for lysine to be 5 7 (18) and of our own in children with nutritional rickets

Investigation of the function of the mucosa is relevant in each of the conditions exem plified here In kwashiorkor there is diar rhoea (27) histological changes in the jejunal epithelium (3 6 25) and impaired activity of disaccharidases (4) Although digestion of protein in the lumen of the gut may not proceed as far as to aminoacids their defective absorption might enhance the effects of low dietary intake On the other hand absorption can be increased (7 15) Aminoacids par ticularly alanine (1) in the blood quickly rose when milk was introduced to children with kwashiorkor and overflowed in the urine (23) In observations using methods similar to these gut slices from rats on a diet containing no prot in took up more lysine and glycine than those from animals on a 5% protein di t (which had produced a kwashiorkor like syndrome) or from controls on a full diet (16) In the determinations reported here however neither depression nor enhancement of mucosal absorptive activity was demonstrated in the actual human situation

Bacteria and mucosa interact germ free animals have more regular villi wider brush border and better absorption of xylose than normal (14) while in tropical sprue normal villi are decreased and micro-organisms in creased (8) Repeated attacks of acute gastroenteritis occur in association with malnutri tion and their effects may summate with it (24 28) Malabsorption of sugars may be found Absorption of aminoacids is important for although oral intake of protein may be low endogenous loss into the bowel may be considerable in secretions exudate blood and sloughed cells (19) However our findings do not confirm the expectation of inadequate up take in gastroenteritis

The effect of neomycin is of importance be cause although a known cause of malabsorption (2 9 20) and histological change (12) it is much used in the treatment of gastroenteritis In this study neomycin was given in standard therapeutic dose to patients who were vulnerable in that they were malnou rished and had intestinal disease Though the ratios were rather variable it was not shown to inhibit absorption

Giardia lamblia live in the mucus of the small intestine in close association with the mucosa which they may invade (5) It is often found in malnourished children (3) and was seen in eight of our cases The protozoan may cause acute diarrhoea or chronic malabsorption (12 21) but it may be asymptomatic and its usual clinical significance is uncertain The present of giardia was not associated with low D R s and no direct effect on the mucosa has been demonstrated here

Intolerance of milk caused by low lactase activity in the epithelium was found in five members of this series Absorption may not be affected of substances other than lactose water and electrolytes cases of kwashiorkor with intolerance can be cured with milk alone These results were not at variance with this clinical observation and did not show impair ment of aminoacid uptake in the children who had large acid stools while on milk

Repetition of these studies with material from more seriously ill patients in different experimental conditions or with other aminoacids might produce other results but no evi dence has been found that there was a change in aminoacid handling in these diseases

SUMMARY

The uptake in vitro of lysine and alanine by jejunal mucosa was investigated in kwashi orkor acute gastroenteritis and after neomycin therapy Some of the children also had giardia sis and lactose intolerance No consistent evidence of abnormality was found in any of these diseases under the conditions of the study

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PLASMA AND ERYTHROCYTE AMINO ACIDS DURING TREATMENT OF PROTEIN CALORIE MALNUTRITION

C N U Report 56

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Our earlier studies on plasma and erythrocyte amino acids in malnutrition (6 7) revealed the surprising fact that the red cells in severely malnourished children often showed increased levels of amino acids in spite of very low plasma values resulting in high erythrocyte/plasma distribution ratios for most of the amino acids studied The effect of dietary treatment of severe protein calorie malnutrition on the erythrocyte/plasma ratio for valine and isoleucine–leucine was followed in a pilot study comprising 6 cases The present investigation comprises 32 malnourished children admitted to the Ethio-Swedish Paediatric Clinic in Addis Ababa during 1965 and 1966 The purpose was to follow the effect of standardized dietary treatment on individual amino acids in plasma as well as in red cells and to further assess the possible clinical value of erythrocyte/plasma amino acid ratios

MATERIAL

Twenty nine out of the 32 children were between 9 and 36 months of age The 3 remaining children were aged 4 5 and 7 years respectively Sixteen children were boys and 16 girls All children suffered from protein calorie malnutrition of the kwashiorkor type They had all reduced weight for age according to the Harvard standard Thirty children were in the range of 45–70 and the remaining 2 children had a weight for age of 80% of this standard Privileg d Ethiopian children have a weight curve very close to the Harvard standard (17) All children had muscl wasting oedema and other typical symptoms and signs of severe protein calorie malnutrition Reduction of serum albumin was present in all cases and most of the children had a moderate anaemia Several of the children suffered from infections at admission or shortly before admission mostly diarrhoea respiratory infections or measles

Th majority of the cases were admitted from Addis Ababa. The dietary histories revealed very unsatisfactory diets during and after the weaning period (21) Usually no animal protein whatsoever was included in the diet. In the few cases when milk was used the quantities were very small The dietary treatment was started on th day after admission when the first sample of blood was taken During the first week no food other than skim milk was given usually 900 g/day The milk was prepared from dry skim milk Thereafter when the second blood sample had been taken a mixed diet including skim milk was given A third blood sample was taken when the children had been on the mixed diet for 12 to 16 days During th first week the diet thus provided 30 g of protein and negligible amounts of fat per day irrespective of age and weight of the child Exact figures for the mixed diet introduced from beginning of the second week were not available but it contained more protein than during the first period and also veg table fat. Eight of the children died during the first week after admission 24 children recovered

A group comprising 12 orphanage children, 9 children from socioeconomic group I and 7 village children all apparently h althy and between 1 and 10 years of age was used as control material for plasma amino acids No significant differences were observed between the subgroups and the material has therefore been used combined Erythrocyte amino acid

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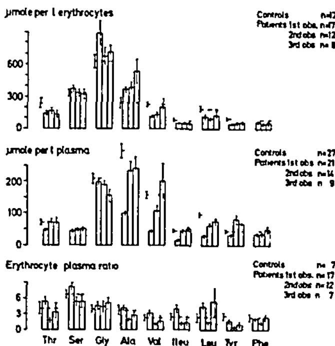


Fig 1 Erythrocyte and plasma amino acid concentrations and erythrocyte/plasma distribution ratios in children with advanced protein calorie malnutrition Blood sample obtained on admission (first column) and after one (second column) and 2-3 weeks of treatment (third column) Horizontal dotted lines show the mean values in the control material and the bars indicate the S E M

values were available only for the 12 orphanage children

METHODS

Fasting blood specimens were collected on the day after admission after 1 and after 3 weeks of treatment The specimens were prepared for amino acid analysis according to the following technique The blood was drawn into a weighed heparinized tube and mixed carefully The haematocrit value was determined in duplicate The tube was weighed again and the weight of the blood specimen calculated by difference After centrifugation plasma and erythrocytes were separated The separation of the red cells from plasma was completed within 15 min after the blood specimen had been drawn Portions of 2 ml plasma were deproteinized with 10 ml 1 picric acid

The red cells were washed twice with 5 ml aliquots of saline as previously described (5) During this procedure no red cells were lost The packed red cells were haemolysed by adding 0 005 M phosphate buffer pH 7 0 to a final volume of 15 ml After centrifugation the proteins were precipitated by addition of 75 ml 1 % picric acid The whole procedure including the precipitation of proteins was completed within 1 hour after the blood specimen had been drawn

The picric acid filtrates from plasma and red cells were frozen and sent to Sweden for amino acid analysis according to the method of Spackman et al (16)

METHOD OF CALCULATION

The amino acid concentration in the red cells was calculated according to the following formula

µmole amino acid per litre of erythrocytes =

$$\frac{A \times B \times C \times 100}{D \times E}$$

where (A) is the amino acid concentration in the haemolysate (µmol/l)

(B) is the specific gravity of the blood sample (assumed to be 1 05)

(C) is the final volume of the haemolysate (15 ml)

(D) is the weight of the blood sample (g)

(E) is the haematocrit value of the blood sample (vol%)

RESULTS

The following amino acids were analysed serine, threonine glycine alanine valine, isoleucine leucine tyrosine and phenylalanine The material has been divided into two groups

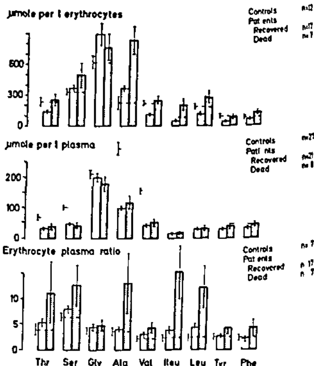


Fig 2 Initial erythrocyte and plasma amino acid concentrations and erythrocyte/plasma distribution ratios in children with advanced protein calorie malnutrition a comparison between those who recovered (◻) and those who died (◼) Horisontal lines show the mean values in the control material and the bars show the S E M

the first including those cases who recovered and the second those who died From those who died only one blood sample was obtained

In those who recovered the plasma levels of most of the amino acids studied were low in the sample taken on admission as compared with the controls (Fig 1) Significantly lower values were obtained for alanine valine isoleucine and leucine Already after 1 week of treatment most amino acid levels were normalized although in some cases there was a further increase during the second and third weeks of treatment The initial erythrocyte amino acid levels in cases who recovered were mostly within the range of the control material or somewhat lower (valine tyrosine) and did not change significantly during treatment Most of the cases who later died (Fig 2) however had high erythrocyte amino acids The mean values for alanine valine isoleucine and leucine in this group were significantly higher than in the group of children who recovered The alanine value was even higher than in the control group Since these amino acids were all low in plasma before treatment very high erythrocyte/plasma ratios were obtained The difference in erythrocyte amino acids between children who died and recovered did not correspond to any difference in plasma amino acids between these groups

DISCUSSION

All amino acids except glycine showed reduced values in our cases of protein calorie malnutrition This is in good agreement with other studies (2 5 11 17 18 20) The glycine concentration in our cases was normal Holt et al (14) however reported abnormally high glycine values The glycine level in their control material consisting of 8 American children was however considerably lower than in our control group consisting of 27 apparently healthy Ethiopian children The normalization of the plasma amino acids within the first week of treatment is in accordance with the findings of Whitehead & D an (19) Arroyave & Bowering (1) on the other hand report a more slow normalization of the plasma amino acid pattern Also the reverse effect denormalization on a diet with unbalanced amino acid composition has been observed (4 15) It seems logical therefore to compare diets by evaluation of the normalization and denormalization of plasma amino acid levels in children Such experiments are now in progress using different types of so-called protein rich foods or weaning foods for infants as test material

The erythrocyte amino acids seem generally not to decrease even in severe cases of malnutrition On the contrary increased values were observed in many cases This indicated a serious prognosis Of the eight children who died only one had normal erythrocyte amino acid levels on admission and among those who recovered elevated levels were seen only in two cases This verifies our previous (7) observations of high erythrocyte/plasma ratios in severe cases of malnutrition As has been demonstrated by Bjornesjo et al (3) high erythrocyte amino acid values are observed also in a variety of infections and other diseases It is therefore probable that the present findings of increased red cell amino acids should be interpreted as side efects of concomitant infections rather than effects of the protein malnutrition per se As shown by Carlsten et al (8 9 10) and later verified by Felig et al (13) alanine has a key role in the ammonia transport and the gluconeogenesis Even during starvation there is a continuous formation of alanine by transamination of pyruvate at least in muscle cells An increased intracellular formation of alanine might explain the high content of this amino acid in the red cells The elevated level of alanine might reflect either an increased supply of pyruvate or an increased supply of the amino acids providing the amino group

SUMMARY

The plasma and erythrocyte amino acid concentrations were determined in 32 children

with advanced protein calorie malnutrition The previously well documented decrease in the plasma levels of most of the amino acids was confirmed The glycine concentration was not altered High erythrocyte amino acid levels were a serious prognostic sign, since 7 out of 9 children with increased amino acid levels in erythrocytes died In those children who recovered there was a normalization of the plasma amino acid levels during the first week of treatment

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NUTRITION IN LOW BIRTH WEIGHT INFANTS

I *Intravenous Injection of Fat Emulsion*

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In the low birth weight (LBW) infant the supply of sufficient amounts of calories is important The estimated caloric need for a LBW infant is some 60–80 kcal/kg b w and 24 h at neutral temperature (9 10 15) When respiratory distress or lowered environmental temperature are added the caloric requirements increase considerably Immaturity and respiratory distress are often obstacles to oral feeding Infusion therapy supplying fluid and calories to LBW infants decreases catabolism (1) and reduces mortality in the neonatal period (3) A daily supply of 65 ml/kg b w of fluid as a 10% sugar solution is a commonly adopted procedure but supplies only approximately 25 kcal/kg b w or about one third of the actual need

The logical choice in the search for a more sufficient parenteral supply of calories is fat which gives 9 kcal/g Intralipid® (Vitrum Stockholm) an isotonic fat emulsion of soy bean oil and lecithin has been used extensively for nutritional purposes in adult man The kinetics of injected and infused fat of this particular type of emulsion have been carefully studied in the dog and in adult man (7) Single injections of Intralipid® have been given to newborn infants without apparent side effects (21) The fat particles of Intralipid® have physical characteristics resembling those of native chylomicrons and are metabolized through the same pathways (7)

The aim of the present study was to investigate the kinetics of single injections of Intralipid® fat emulsion in LBW infants

MATERIAL AND METHODS

Clinical material

Forty two low birth weight (LBW) infants with birth weights of 2 500 g or less were studied No infants with respiratory distress malformation or other deviation from the normal except dysmaturity and/or immaturity were included The birth weight and gestational age of each infant was plotted (Fig 1) This figure also displays the relationship between birth weight and gestational age in a large Swedish population (4) The material was divided into the following three groups *A pre term infants* above the 10th percentile (21 infants) *C light for-dates* below the 3rd percentile (14 infants) and *B* an *intermediate group* of infants located between the two lines (7 infants) These terms will be used throughout the text

Injection procedure

Fat emulsion as Intralipid® (20% soybean oil 1.2% lecithin and 2.5% glycerol) was given as a single injection through an umbilical vein catheter with its tip in the inferior vena cava

Seventeen infants were given 0.1 g fat/kg b w in 1 min and another group of 25 infants 0.5 g fat/kg b w in 5 min The fat injection was followed by an intravenous infusion of 0.9% saline or 10% Invertos (5 g glucose plus 5 g fructose per 100 ml) In 34

These results were presented in part before the Swedish Paediatric Society Goteborg, April 1969 and the European Society for Paediatric Research Stockholm August 1970

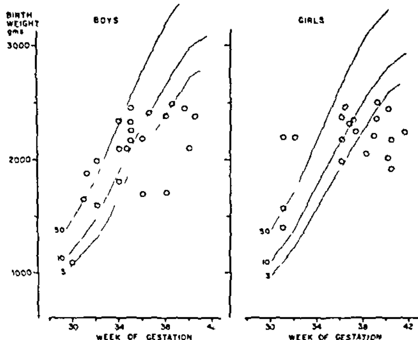


Fig 1 Distribution of the material according to birth weight and gestational age The curves indicate the 50th 10th and 3rd percentile of a Swedish reference material (4)

infants the fat was injected before the age of 6 hours and in the remaining 8 infants before the age of 24 hours The infants were not fed orally before or during the time of the study

Blood sampling and variables studied

Blood samples were drawn from the umbilical catheter after careful rinsing to avoid admixture with injected fat In the initial phase of the study in addition to serum lipoprotein patterns and total lipids 14 infants were studied with analyses of blood glucose ketone bodies and haematocrit Blood samples for the determination of glucose total lipids and serum lipoprotein patterns were taken at various intervals but usually according to the following scheme Immediately before and after the injection and repeatedly during 2 hours after the injection (6–8 samples) and in some cases also after 6 12 and 24 hours after the injection The differences between pre injection values of total lipids and the total lipid concentrations at 2 hours after the injection were calculated and called delta total lipids (ΔTL) Change in plasma glucose levels within two hours as given in Table 1 was calculated as the average of blood glucose values after the infusion (up to 2 hours) less the initial value

The removal rate of the Intralipid® particles was studied by plotting the elimination curves and the ΔTL values at 2 hours after injection The elimination of injected Intralipid® can be characterized by a two phase system as described by Hallberg (7) Figure 2 (from Hallberg's work) shows that above a critical concentration the removal rate is constant (λ) and below that concentration the removal rate is fractional (λ_2) The dimensions of the constants are for λ_1 mg/(100 ml × min) and for λ_2 per cent/min

The total amount of blood drawn from each infant varied between 15 and 20 ml within 2 to 24 hours

Chemical methods

Total lipids were assayed by the method of Zollner & Kirsch (22) using sulfo vanillinic acid as a reagent and allowing the use of 50 µl per sample The method was calibrated for Intralipid® and for serum (factors 0 50 and 0 70 respectively)

The method was validated against a reference method (calculation of the sum of spectrophotometrically measured individual lipid fractions) Using the factor 0 70 in neonatal plasma the sulfo vanillinic acid method overestimated the values of the reference method by 18 ± 2 7 per cent (S E M) (n = 26)

Glucose was determined in serum samples by a glucose oxidase method (12) and blood glucose concentrations estimated using the simultaneously determined haematocrit The glucose content of the erythrocytes was neglected Haematocrit was obtained by a capillary tube centrifugation technique Ketone bodies as aceto acetate were determined by a micromethod according to Walker (20) allowing the use of 50 µl per sample

Lipoproteins in serum were studied by agarose gel electrophoresis (16)

RESULTS

The single injection of 0 5 g Intralipid®/kg b w was well tolerated in all infants without clinically detectable side effects

Blood glucose ketone bodies and haematocrit are shown in Table 1 for 15 infants before and after the injection of 0 5 g Intralipid® The blood glucose increased within 2 hours in most infants Some infants were hypogluce

Table 1 *Change in blood glucose ketone bodies and haematocrit levels after the fat injection in fifteen low birth weight infants*

Injection dose 0 5 g Intralipid®/kg b w

Category	Case no	Subsequent infusion NaCl	Subsequent infusion Invertose	Blood glucose Before injection	Blood glucose Change within 2 hrs	Ketone bodies Before injection	Ketone bodies Change after 6 hrs	Haematocrit Before injection	Haematocrit Change after 6 hrs
Pre term	4		×	98	—	455	—	40	−8
	5		×	80	−15	90	+355	55	−8
	7		×	51	+26	—	—	55	−8
	16			17	+9	181	−147	54	−4
	17		×	33	+29	724	−362	55	−8
	$\bar{X}$			56		363		52	
Intermediate	3			100	+3	492	−91	60	−10
	12		×	8	+480	2 280	−647	48	−6
	13		×	13	+4	2 280	−429	61	−3
	14		×	13	+40	376	−218	60	−2
	$\bar{X}$			59		1 308		56	
Light for-date	6			40	+129	700	−560	55	−8
	8			132	−8	362	—	53	−1
	9			91	+114	269	−178	47	−22
	10			73	+68	269	−54	55	−7
	11			14	+83	215	−147	64	34
	15			30	+10	223	−37	56	+6
	$\bar{X}$			63		340		55	

mic at the time of injection The concentration of ketone bodies was increased 6 hours after the injection in 2 infants but decreased in ten The haematocrit showed a slight reduction in most cases

Total lipid and lipoprotein patterns

0 1 g Intralipid®/kg b w given This smaller dose of Intralipid® was given to 17 infants The results are presented in Table 2 On serum lipoprotein electrophoresis the chylomicrons (Intralipid® particles) were demonstrable in

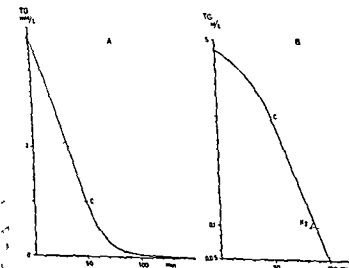


Fig 2 Definition of K and K as demonstrated by elimination of triglycerides (TG) in the dog after a single injection of Intralipid® A shows the linear elimination rate corresponding to K above a critical concentration (C) and (B) demonstrates the fractional elimination rate (K) below the critical concentration (From Hallberg D Elimination of exogenous lipids from the blood stream *Acta Physiol Scand 65* Suppl 254 1965 ref 7)

Table 2 *Initial serum total lipids maximal removal capacity (K_1) and fractional removal rate (K_2) serum lipoprotein electrophoresis on agarose gel in seventeen infants*

Injection dose 0 1 g/kg b w

Category	Case no	Initial serum total lipids mg/100 ml	K_1 (mg/100 ml/min)	K_2 (/min)	Electrophoresis (minutes after injection when last observed)	
					Chylomicrons	Pre β lipoproteins
Pre term	29	507	6 5	—	20	40
	34	472	12 3	5 3	20	Never observed
	35	233	—	4 6	20	Never observed
	36	462	—	3 3	20	Never observed
	37	277	14 4	9 3	0	Never observed
	40	252	—	8 6	30	Never observed
	41	274	12 0	4 3	30	Never observed
	42	273	—	—	40[a]	Never observed
	43	175	10 0	6 9	20	Never observed
	44	233	7 4	5 3	10	Never observed
	$\bar{X}$	315 0	10 4	6 0		
Intermediate	28	511	—	3 1	20	60
	33	362	6 3	—	20	Never observed
Light for date	30	364	10 2	4 3	120[a]	120[a]
	31	360	17 0	4 9	40	120[a]
	32	386	—	3 3	40	120[a]
	38	301	7 5	—	60	120[a]
	39	305	4 7	3 1	45[a]	45[a]
	$\bar{X}$	343 2	9 9	3 9		

[a] Last samples

only one of the pre term infants in samples taken later than 30 min after the fat injection In the light for dates the Intralipid® was still present 40 to 120 min after injection in all cases In these infants no samples were taken after 120 min Pre β lipoproteins (pre β LP) appeared after the injection only in one out of 10 infants in the pre term group but occurred in all the light for dates

0 5 g Intralipid®/kg b w given Values for total lipids before and after the fat injection are presented in Table 3 Total lipid concentrations versus time after the injection are plotted in Fig 3 There are no significant differences in pre injectional or in peak concentrations (highest total lipid value after the injection) between pre term and light for dates The pre terms however, cleared their plasma faster than did the light for dates (Fig 3) ΔTL values at 120 min after the injection were significantly different in the two groups on 0 01 significance level (Table 3), the means were 22 mg/100 ml and 186 mg/100 ml respectively

From Fig 1 the percentage deviation of weight from the 50th percentile for each infant was calculated and plotted against the ΔTL values at 120 min (Fig 4) The figure demonstrates the correlation between high ΔTL values at 120 min and marked under weight in relation to expected birth weight This relationship is statistically significant ($p < 0\,01$) A hyperbolic regression line was fitted to the material giving the equation

$$\sqrt{y + 100} = 9\,58 - 0\,17X$$

where y = ΔTL at 120 min and X the deviation from the expected birth weight expressed in per cent

One group of infants obtained an infusion of Invertose after the injection of Intralipid® whereas another group was infused with saline The two groups are marked separately in Fig 4 No apparent difference in removal capacity between the groups was observed Neither was

Table 3 *Difference in total lipid level at 120 min after injection and the initial level and initial and maximal total lipid levels in twenty five infants*

Injection dose 0 5 g Intralipid®/kg b w

Category	Case no	Subsequent infusion		Initial total lipids (mg/100 ml)	Maximal total lipids (mg/100 ml)	ΔTotal lipids after 120 min
		NaCl	Invertose			
Pre term	4		×	250	—	—
	5		×	328	1140	0
	7		×	392	784	29
	16	×		214	695	0
	17		×	278	785	36
	18			333	949	0
	19			311	580	0
	20	×		234	1496	21
	22	×		175	622	25
	25	×		195	1073	97
	27			314	914	15
	$\bar{X}$			275	904	22
Intermediate	3	×		115	1785	385
	12		×	226	726	0
	13		×	256	769	0
	14		×	223	670	17
	23	×		260	866	0
	$\bar{X}$			216	963	80
Light for date	6		×	363	939	90
	8			233	688	167
	9		×	225	825	150
	10		×	447	868	92
	11		×	409	1136	330
	15		×	175	766	470
	21			193	604	0
	24			188	915	260
	26			235	764	113
	$\bar{X}$			274	834	186

there a difference when the regression lines for the two groups were compared

Lipoprotein patterns before and after the injection of 0 5 g Intralipid®/kg b w are shown in Figs 5 and 6 for one representative pre term and one light for-date infant respectively The difference between the two groups in the occurrence of a pre β LP fraction as described above after the injection of 0 1 g Intralipid®/kg b w is clearly demonstrated The lower disappearance rate of the chylomicrons (Intralipid® particles) in the light for-date infants is also apparent Fig 7 demonstrates for the whole material these differences in post injectional lipoprotein patterns after the injection of 0 5 Intralipid®/kg b w The evaluations of the electropherograms were performed without access to the birth weight data In Fig 7 the infants were arranged according to percentage deviation from the expected birth weight in relation to gestational age The figure demonstrates that there is a connection between relative underweight and a lower removal rate of injected fat particles combined with an increased occurrence of pre β LP

Maximum removal capacity (K_1) and fractional removal rate (K_2) of the chylomicrons (Intralipid® particles)

Utilizing the results at the injection of 0 1 g Intralipid®/kg b w in 17 LBW infants the K_1 value the maximum removal capactiy and the K_2 value the fractional removal rate were calculated In 11 cases the graphs allowed the calculation of K_1 and in 13 cases K_2 could be deduced The difficulty encountered in study

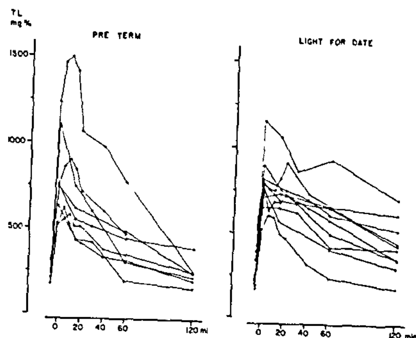


Fig 3 Elimination curves in pre term and light for-date infants after i v load of 0 5 g Intralipid®/kg b w

ing the K values was the early appearance of pre β LP The values for K_1 ranged between 5 and 17 mg/(100 ml × min) and the K values were about 5 per cent/min (Table 2) The means of K_1 and K_2 values differed although not significantly between pre term and light for date infants

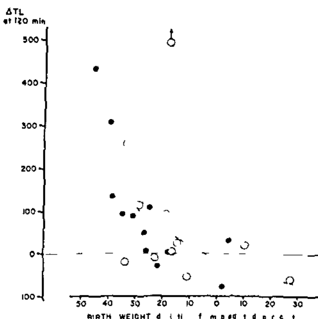


Fig 4 Delta total lipids (ΔTL) at 120 min after injection of 0 5 g Intralipid®/kg b w as a function of percentage deviation of birth weight from the 50th percentile of birth weight in relation to gestational age Filled circles represent fructose glucose infusion and open circles 0 9 per cent saline infusion given after the fat injection The dotted line represents the equation

$\sqrt{y+100} = 9\ 58 - 0\ 17X$

DISCUSSION

In this study two different doses of fat have been used When 0 5 g Intralipid®/kg b w was given the shape of the elimination curve was disturbed by the appearance of a second generation of lipoprotein particles A dose of 0 1 g/kg b w on the other hand resulted in disappearance curves that in several cases could be analysed as described by Hallberg (7) in man and in the dog A K_1 value i e maximum removal capacity was calculated for 11 infants to be between 5 and 17 mg/(100 ml × min) Due to the early appearance of a second generation of lipoprotein particles the estimations of K values were more difficult In 13 infants the disappearance curves allowed the calculation of K The K ranged between 3 and 9 per cent/min These K_1 and K values were in good agreement with those given for adult man (7) The half life corresponding to a K value of 5 per cent/min was 14 min This should be compared with the normal half life for native chylomicrons of 17 min as given by Hallberg (7)

The graphs on the removal of exogenous fat

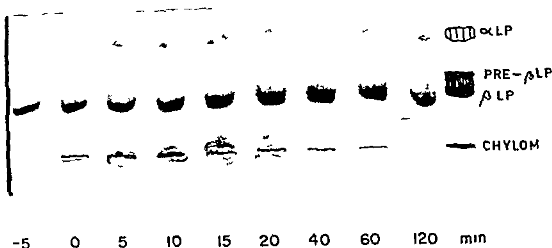


Fig 5 Photograph of an electrophoretic analysis in agarose gel of serum lipoproteins from a pre term *infant* with birth weight deviation of +27% of the *50th percentile* of birth weight in relation to gestational age Injection dose 0 5 g Intralipid®/kg b w The chylomicron fraction (Intralipid® particles) is present at 40 min and a trace at 60 min but is not observed at 120 min A trace of pre β LP could be seen at 40 and 60 min

(Fig 3) in combination with the electrophoretic lipoprotein patterns indicated the appearance of new lipoproteins already within 10 min This suggested that the injected triglycerides which had already left the intravascular compartment at this time were re introduced

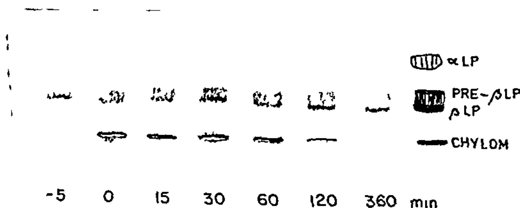


Fig 6 Photograph of an *electrophoretic* analysis in agarose gel of serum lipoproteins from a light for date infant with birth weight deviation of −39% of the 50th *percentile* of birth weight in relation to gestational age Injection dose 0 5 g Intralipid®/kg b w The chylomicron fraction (Intralipid® particles) persists up to 120 min and a definite increase in pre β LP occurs early after the injection and is still detectable at 120 min

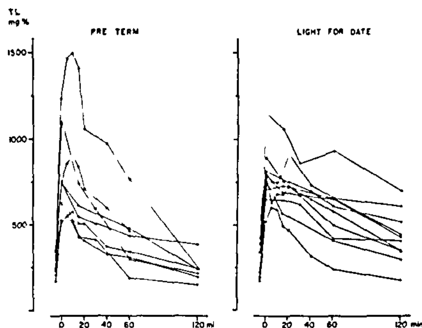


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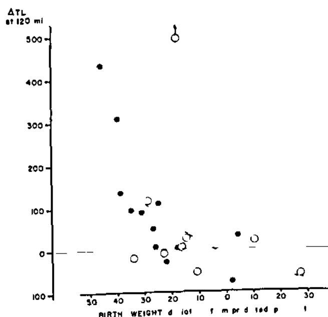


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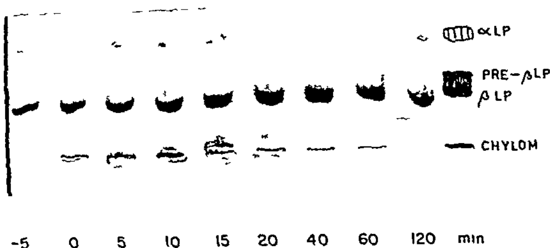
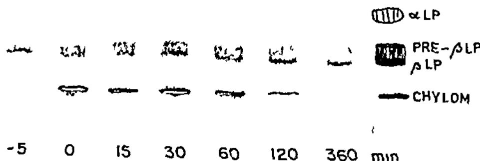


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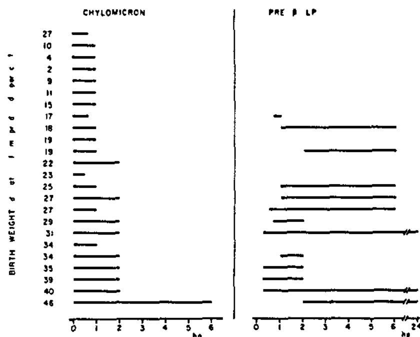


Fig 7 Presence of chylomicrons (In tralipid® particles) and pre β LP afte a fat load of 0 5 g Intralipid®/kg b w The cases are arranged according t their percentage deviation from pre dicted birth weight (50th percentile o birth weight in relation to gestationa age)

into the circulation These additional lipids appeared on agarose gel electrophoresis as pre β LP, presumably released from the liver (Figs 5 and 6) When pre β LP were present in excess the exogenous fat the Intralipid® particles disappeared from the plasma at a lower rate (Fig 7) The appearance of pre β LP was absent or slight in the pre terms but pronounced in the light for date infants (Figs 5 6 and 7)

The results of the present study demonstrate that a quantitative difference in the metabo lism of exogenous fat exists between the two groups of LBW infants studied the pre term infants and the light for dates Whether this is due to differences in the intrauterine nutrition or gestational age cannot be deter mined with certainty from the available data It is however less probable that an increased maturity would lead to a reduced removal rate of triglycerides Our present view, of a lower removal being due to intra uterine under nu trition is also supported by the findings of Melichar et al (14) These authors showed after an oral load of olive oil 0 5 g/kg b w a significantly greater rise in plasma esterified fatty acids for hypotrophic as compared to normal full term newborns

A reduced rate of triglyceride clearance in the light for date infants may be due to inel ficient lipoprotein lipase activity In infant with marasmic extra uterine malnutrition, subnormal lipoprotein lipase response has bee shown after heparin injection (6)

Exogenous triglycerides as chylomicron and also newly synthesized triglyceride m pre β LP are considered normally eliminat through the uptake of their free fatty ac moiety by the adipose tissue or through tl uptake of the intact lipoprotein particle l the liver (8)

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The uptake of free fatty acids in the adi pose tissue also depends on the adequate form ation from glucose (8) of alpha glycero phos phate In the light for date infant a sufficien amount of glucose for the esterification o

fatty acids in the adipose tissue may not be available In the present work the role for the triglyceride removal of a simultaneous glucose supply was tested No apparent difference in the rate of triglyceride elimination was found in infants given fructose glucose and those given a saline infusion (Fig 4 and Table 3)

One earlier study has suggested an inappropriately high insulin secretion in light for-date infants (17) An increased level of plasma insulin would stimulate lipoprotein lipase activity and rather favour the fatty acid uptake in the adipose tissue On the other hand recent data suggest that a high plasma insulin level would increase the liver synthesis of triglycerides and the output from the liver of pre β LP (17) These newly synthesized pre β LP may compete with the exogenous fat for elimination capacity However in a recent careful study Gentz et al (5) were unable to demonstrate a difference between pre terms and light for-dates with respect to basal insulin secretion or insulin secretion after a glucose load Therefore it appears possible to exclude an inappropriate insulin secretion as a major cause for the observed difference in lipid elimination

SUMMARY

Intravenous fat loads were given to low birth weight (LBW) infants The fat was administered as 20 % Intralipid® *(a soybean oil emulsion)* as a single injection of 0 5 or 0 1 g/kg bodyweight (b w) The concentration of total lipids was followed in serum samples and the serum lipoproteins were characterized by means of agarose gel electrophoresis *In* pre term infants the maximum removal capacity of fat from the intravascular compartment averaged 10 mg/(100 ml × min) In light for-date infants the initial maximum removal capacity was of the same order but the removal rate decreased concomitant with the appearance in the blood stream of a second generation of lipoprotein particles These were identified by serum lipoprotein electrophoresis on agarose gel as pre β lipoproteins and were considered to originate in the liver The increase of total lipids remaining 120 minutes after the injection of 0 5 g Intralipid®/kg b w (ΔTL at 120 min) gave a significant regression line when expressed as a function of the deviation from predicted birth weight The maximum removal rate of exogenous triglycerides given as Intralipid® was in the pre term infants within the range of that found in normal adults while in the light for-date infants a lower overall removal capability was found

ACKNOWLEDGEMENTS

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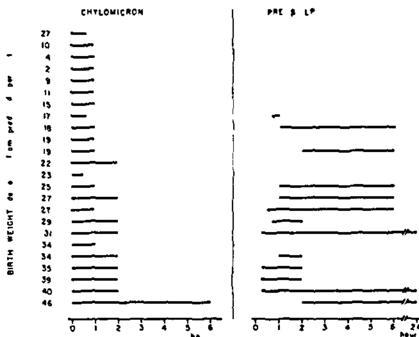


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CHANGES IN SKIN TEMPERATURE OF THE NEONATE AT BIRTH

A cine thermographic study

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From the Radiodiagnostic Department of Meilahti Hospital the Midwifery Institute Helsinki Finland and the Wenner Gren Cardiovascular Research Laboratory Norrtull's Hospital Stockholm Sweden

The principal mechanism of controlling heat loss during the first postnatal hour is the constriction of skin vessels thus reducing radiation and the temperature gradient between skin and environment (2 4) Because of lack of suitable methods our knowledge is only fragmentary concerning the immediate response of the peripheral circulation at birth

Thermography offers a method to map out the heat distribution of the skin and by cine thermography sudden changes in temperature can be recorded almost continuously

METHODS

An AGA Thermovision model 661 was used The equipment is made up of two basic units an infrared camera and a modified oscilloscope that displays the thermal picture on its screen The relation between temperature and radiation intensity is highly non linear Therefore the instrument s calibration can be directly interpreted in terms of temperature only for a restricted range of temperature around the calibration temperature The range is 20 C to 40 C for the instruments used (5)

The camera equipment used was a Bolex 16 mm cinecamera fitted with a 50/60 Hz synchronous motor The gear ratio has been chosen so that every other thermovision picture is filmed i e the cine frame rate is 8 frame second The equipment also includes a phasing device so that frame begins to be exposed at the same moment as the thermovision picture sweep commences

Supported by the Swedish Medical Research Council Stockholm Sweden, and the Association for the Aid of Crippled Children New York, USA

The camera was placed in the delivery room about 2 m from the labour bed The thermograph was turned on at least 15 min before expected labour in order to reach satisfactory stability of the equipment The temperature of the delivery room was about 24 C The recording began at the moment when the vertex first appeared In all fullterm babies skin temperature of the center of both palms and heels, epigastrium and back (between the scapulae) were measured with an electrical thermometer type Sekunden Thermometer using skin applicator The response time of this electrode was 2–3 sec Registrations were made within 10 sec at 1 3 and 5 min after birth in order to get a rough control of the temperature changes seen on the thermal picture and of the degree of cooling during the first 5 min In a few infants temperatures of the skull cheeks and nose were also measured with the skin applicator

MATERIAL

Twelve healthy fullterm infants of normal birth weight were studied The infants were given a mean Apgar score of 9–10 at 1 and at 5 min They all began to cry within 10 sec Two healthy premature babies were included in the material Their Apgar score was 8 (1 min) and the birth weight 1 800 and 2 200 g respectively In both cases the adaptation was considered normal Besides two asphyxiated babies with a birth weight of 2 600 and 1 800 g were studied At 1 min Apgar score was 4 and 5 respectively These babies were resuscitated and survived Within 15 days they left the maternity hospital without any signs of abnormality

RESULTS

The following observations were made from the cinethermographic films of the normal

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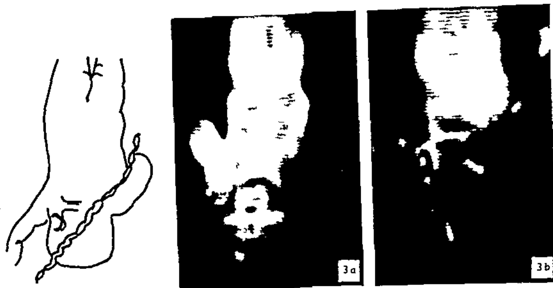


Fig 3 (*a*) A neonate is held upside down about 15 sec after birth The front, cheeks and chin have cooled off Note the cold nose The temperature of the whole left arm has now dropped Again the cool patches on the thorax appear with the first breath

(*b*) The same baby turned around about 30 sec later The skull the earlobe the nape both arms and the umbilical cord are much colder than the trunk No dark areas corresponding to those on the anterior surface of the trunk are seen

cedaneum had a skin temperature of only 34 C over this area already before the head was cutting through

When the *face* appeared the temperature of the cheeks and nose dropped from about 36 to about 34 C within 10 sec This fall of temperature caused a change from white to black on the thermal picture

As soon as the arms appeared their skin began to cool off Either surface temperature over the whole arm dropped immediately or the cooling started from the hand proceeding in a centripetal direction A few moments later the same pattern of cooling was observed on the legs

With the first deep breath there was an instantaneous drop in skin temperature of the area on the anterior thoracic cage corresponding to the lungs (Figs 2 and 3) No such reaction was seen on the posterior thorax region Cooling of the skin on the back was slower and irregular thus patches with lower temperature than surrounding areas appeared here and there (Fig 3 *b*) Vernix when present delayed the drop in skin temperature

The highest heat emission was seen over skin folds as for instance in the neck and the ingues

The temperature of the umbilical cord fell instantly in infants born with nuchal cord (4 cases) In the rest of the infants a drop of the umbilical temperature occurred 30 to 60 sec later

The *premature babies* who started to cry immediately showed the same pattern of reactions as the fullterm infants

The infants with *asphyxia pallida* behaved in a different way Cheeks hands and feet cooled down but the whole trunk surface remained relatively warm (around 35 C) Cooling of the skin over the thoracic cage probably did not occur until respiration was established Attempts to initiate respiration by splashing cold water (about 15 C) over the trunk caused a transient decrease of the heat radiation there (Fig 4)

Shivering was not observed in any infant studied

In three cases the placenta was cinethermographically recorded There was a slow and

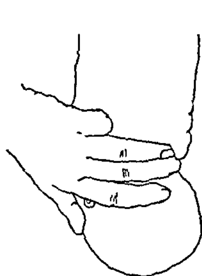
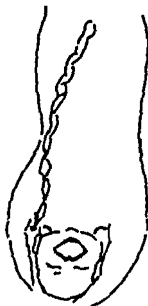






Fig 1 Dark areas are colder than light areas (*a*) The head and trunk are delivered Skin temperature over the head has already dropped The difference in temperature between the trunk and the darkest areas of the skull is at least 1 5 C Note the cold hand of the midwife (*b*) An infant held upside down about 10 se after birth Note the cold area of the skull corresponding to caput succedaneum

fullterm babies The pattern of change in heat emission during the first postnatal minutes was quite uniform in the group of normal infants studied, in spite of minor changes in ambient temperature and air currents of the room When the head was born skin temperature over the skull was 36–37°C During handl of the *head* at delivery areas of lower temperature (about 35°C) appeared on the skull (Fig 1) Children with pronounced caput su





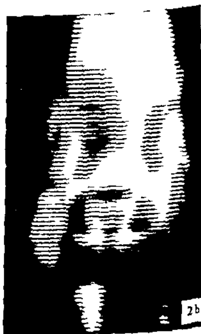


Fig 2 (*a*) A baby held upside down The umbilical cord has cooled off and is well seen against the warm trunk (The baby was born with the cord around the neck) The wide open mouth appears warmer than the surrounding face Peripheral cooling of the upper extremities has just started (*b*) A moment later the baby has taken his first breath colder areas on th thorax corresponding to the lungs appear The coolin of both arms has progressed in a proximal direction

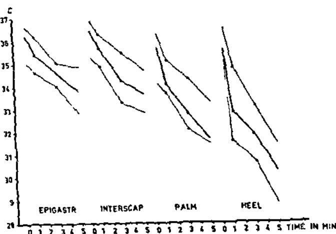


Fig 5 Skin temperatures in 12 infants during the first 5 min of life (Mean Max and Min values)

tory ways represents an important stimulus for chemical thermoregulation Our results indicate further that inhalation of room air (about 24 C) at birth elicits a drop in skin temperature of the thoracic cage This cooling might be explained by a reflectoric vasoconstriction of the skin vessels provoked from the airways

However the same phenomenon was not noted over the posterior thoracic cage perhaps due to difference in thickness of the subcutaneous fat layer and/or in the vasomotor mechanism of this skin area In this connection it can be mentioned that the presence of sheets of brown fat located to the interscapular region of infants has been established (1)

Infants with distinct caput succedaneum have a temperature over the skull of about 34 C already before the head cut through a finding of interest with respect to the Saling technique (8)

The highest skin temperatures were recorded in the skin folds which effectively reduce the radiating surface

The rapid cooling of the umbilical cord in infants with cord round neck might be caused by a constriction of the umbilical vessels during the manipulation of the cord

The asphyxiated newborn infants seemed less able to restrict heat loss right after birth than the infants with a high score This inability might help to explain the disposition of asphyxiated newborn infants for hypothermia

In contrast to the characteristic pattern and rapid decline in skin temperature observed in the infant on the placentas examined the whole surface cooled off in a slow and similar way perhaps due to the lack of nervous vasomotor control

SUMMARY

Cinethermography permitting a continuous recording of heat emission of wide areas of the baby was used to study the newborn infant s first reaction to a cold environment Examination was carried on from the moment of birth through the first minutes of life and controls of temperatures with skin applicator were made A rapid drop of temperature of the extremities starting from the periphery and quickly advancing in centripetal direction was noticed As air entered mouth airways and lungs a precipitous fall in temperature over the corresponding anterior thorax areas occurred No similar drop on the posterior thorax region was seen The highest heat radiation was recorded in the skin folds

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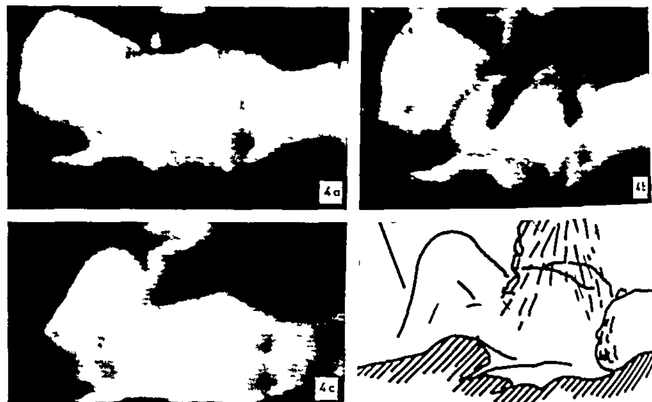


Fig 4 An asphyxiated premature baby recorded be fore any breathing has been established (*a*) The front and cheeks have cooled off whereas the trunk and legs have not (*b*) In order to stimulate respiration cold water (black areas) is sprinkled on the baby Cold spots appear on the trunk (*c*) A few seconds later the trunk and legs are warm again though the head appears colder than on the first two photos

continuous fall in temperature of the surface contrary to the quick cooling seen on the skin of the infants

Temperatures recorded with skin thermo meter are presented in Fig 5 The mean val ues of two measurements at each time are given There is a good correlation to those published earlier (6)

Because of intensive care of the premature and asphyxiated infants no reliable tem perature control measurements with the elec trical thermometer were made

DISCUSSION

At birth the skin temperature drops mo mentarily from the intra uterine level of about 37 5° to 36 5°C which was the highest level we measured anywhere on the surface with the electrical thermometer

The rapid drop of temperature over widely scattered skin areas elicited at birth indicates an effective vasoconstriction which diminishes the dissipation of heat

The rate and extent of fall of skin tempera ture show a variation corresponding to differ ent anatomical regions and demonstrate a characteristic pattern

As soon as air enters the mouth the nose and the pharynx a drop in skin temperature of the nose and cheeks is noted The trige minus region is known to be sensitive to heat and cold Bruck observed vasoconstriction or vasodilatation of the infant s heel during thermal stimulation of the skin of the face (3)

We recorded a quick drop in skin tempera ture in the distal part of the extremities spread ing rapidly in centripetal direction in all healthy infants

Pribylova found a significantly higher oxy gen consumption of babies breathing air of low temperature (5°C) than of babies breath ing air of 36°C (7) She proposed that the thermal impulse on the mucosa of the respira

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THE CONSERVATIVE MANAGEMENT OF INFANTILE HYDROCEPHALUS

B HAGBERG and A S NAGLO

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There are still several unsolved and intriguing problems concerning the best method of management in the individual case of infantile hydrocephalus The easiest way for the doctor is to decide immediately after diagnosis to devise a shunt for diverting the accumulating amounts of CSF However it is a general experience today that all these surgical procedures are subject to numerous complications in the form of different sorts of shunt blockage detachments and septic infection In addition with a few exceptions these children have been considered to be dependent on their shunts for the rest of their lives

On the other hand series of untreated hydrocephalic children (3 4 6) have clearly shown that no few cases—both within the group of spina bifida cystica and that of simple hydrocephalus—have a clear disposition towards spontaneous arrest after the first years of life At follow up (3 4) some of these patients were found to have a particular brain damage syndrome obviously due to a chronically increased intraventricular pressure in spite of the arrest of abnormal expansion of the skull Other patients—though relatively few—were found to be in perfect mental motor and neurological condition

The most difficult problem seems to be that of establishing distinct criteria for differentiation during early infancy between patients who must be operated upon to avoid brain damage and those who can be left without shunting The present series of 45 thoroughly selected children comprises that part of a 10-year clinical material for whom we chose—on the basis of experiences from an earlier 100 % untreated group (4) and with success or failure—to avoid a shunting procedure altogether or for at least 6 months from the time of diagnosis

CLINICAL MATERIAL AND METHODS

During the 10-year period 1960–1969 179 infants with a diagnosis of infantile hydrocephalus were cared for at the Departm nt of Paediatrics in Uppsala In 135 patients th re was simple hydrocephalus and in 44 the hydrocephalus was associated with spina bifida cystica In altogether 118 cases a Spitz Holter shunt procedure was performed Of the remaining 61 patients 21 were excluded from the pres nt material either because they did not fulfil reasonable criteria for b ing considered to have a tru expanding hydrocephalus or because they had had such a severe degree of hydrocephalus already at birth and such incapacitating malformations of other organs that every form of treatment had b en immediately withh ld

Forty children (11 girls and 29 boys) not subjected to operation thus remained wh re from the development and degree of their hydrocephalus it was considered justifiable not to perform a shunt *immediately* but to wait in the hope of a spontaneous arrest. To this group were added 5 further patients in whom the same cons rvative management was tried for 6 months or more but where a shunt *then had* to be performed i e the original approach clearly failed. The total series thus consisted of 45 patients These

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Table 1 *The clinical non operated material (40 cases) divided according to pathogenetic factors causing the hydrocephalus*

Pathogenesis	Girls	Boys	Total
Hydrocephalus without spina bifida cystica	7	20	27
Aqueductstenosis	0	1	1
Other syndrome of CNS malformation	2	3	5
Basal cisternal block	3	5	8
Pathogenesis unknown[a]	2	11	13
Hydrocephalus combined with spina bifida systica	4	9	13
Arnold Chiari malformation alone	1	1	2
Arnold Chiari + aqueduct stenosis	2	2	4
Pathogenesis unknown[b]	1	6	7
Total	11	29	40

Air-encephalography not performed in [a] 6 cases [b] 7 cases

were divided into two groups A) 31 patients with simple hydrocephalus and B) 14 patients with hydro cephalus associated with spina bifida cystica

The criterion for a diagnosis of expansive hydro cephalus was the presence of a combination of the following two clinical characteristics 1) an abnormally rapid skull growth 2) a dilated ventricular system confirmed either by air encephalography or ventriculography alone (4 cases) echo encephalography alone (13 cases) or a combination of these methods (27 cases) One further patient with obvious hydrocephalus combined with spina bifida cystica was included in spite of the fact that neither air ence phalography nor echo encephalography had ever been performed

The decision to try to avoid a shunt operation was mainly made on simple clinical grounds A slow and only moderately increased deviation of the skull curve at continuous follow up a normal or nearly normal psychomotor development and the absence widened lambdoid sutures all favoured conservat management during the first months of life further support for this decision echo encepha graphy with determination of the echo index acco ing to Sjogren (16 17) was used in several ca Stationary or decreasing indices favoured cons r tive management When the expectancy line had b chosen it must be admitted that it was driven sor times too stubbornly in the hope of reaching a sp taneous arrest within a few months A relatively u form approach was maintained as all patients w seen by one of the authors (Hagberg) during th early period of life

The pathogenetic background to the hydroceph state in the 40 patients not operated on is sho in Table 1 The distribution of the series by age onset and age at diagnosis can be seen in Tabl Of the 5 patients treated with a shunt 4 belon to group A (3 basal cisternal blocks 1 malfor tion) and 1 to group B The final follow up exa nation in the surviving 40 patients was perfor during 1970–1971 The average duration of obse tion had then been 4 5 years (range 1–10 ye The distribution on the series by age at the follow up is shown in Table 3

At the examinations emphasis was placed psychomotor development (Denver and motor

Table 2 *Onset of clinical signs and age at diagnosis*

	Simple hydrocephalus		Hydrocephalus combined with spina bifida cystica	
Age (months)	Onset of clinical signs	Age at diagnosis	Onset of clinical signs	Age at diagnosis
At birth	5			
≤1	7	3	12	7
>1<3	3	8		4
>3<6	6	5		1
>6<9	1	1	1	1
>9<12	1	1		
>12<18	3	3		
>18		6		
Unknown date	1			
Total	27	27	13	13

Table 3 *Age at follow up examination*

Age (years)	Simple hydrocephalus	Hydrocephalus with spina bifida cystica	T[illegible]
1–2	1	1	[illegible]
2–4	7	6	[illegible]
4–6	3	2	[illegible]
6–8	8	2	[illegible]
8–10	4	2	[illegible]
Total	23	13	[illegible]

tests) neurological signs and behaviour deviations The general intellectual level was estimated roughly but no formal intelligence tests were performed Particular watch was kept for symptoms and signs characteristic of the chronic brain syndrome due to infantile hydrocephalus not treated operatively and described in an earlier unselected series of spontaneously arrested hydrocephalus (3 4)

A SIMPLE HYDROCEPHALUS

Mortality

Five of the 31 patients of this group died—4 boys and 1 girl The girl (communicating hydrocephalus due to basal cisternal block) died of meningococcic meningitis when 11 months old One boy (also basal cisternal block) well developed with an apparently arrested hydrocephalus at 7 months of age developed a probable sinus thrombosis followed by severe cerebral complicating defects He died when $4^1/_2$ years old One child had respiratory arrest during an air-encephalographic examination he had to be tracheotomized due to tracheal haemangioma and finally died of secondary bronchopneumonia The fourth child who sustained severe damage from an active cerebral toxoplasmosis and developed secondary brain defects died of a final bronchopneumonia The last case a boy also died of bronchopneumonia when 3 years old and is discussed below among patients in whom a delayed operation had to be performed At least in the first 3 children the fact that a shunt operation had been avoided did not seem to have had any connection with their early death

Delayed operation

Four children had to be operated upon after a period of expectancy of more than 6 months from the time of diagnosis They are regarded as obvious failures in our effort to pick out cases where shunt operations can be avoided Three of the 4 patients had a communicating hydrocephalus secondary to a basal cisternal block The fourth child belonged to the group with cerebral malformation in this case dominated by agenesis of the cor

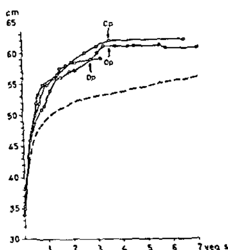


Fig 1 Head circumference curves of the 3 children with simple hydrocephalus operated on later (plotted on the normal curve of Westropp & Barber (19)) --- Upper normal limit (2 S D)

pus callosum He displayed marked hydrocephalus and abnormal neurological features—even at birth In view of experiences from similar cases through the years conservative management was decided upon but was found unsuccessful This severely retarded boy was operated upon when 9 months old to facilitate his care He died of bronchopneumonia at the age of 3 years

The 3 children with basal cisternal block had a rapid skull growth but no other signs of increasing intracranial pressure during their first year of life The abnormal growth of the skull temporarily diminished during their second year of life (Fig 1) but later increased again The psychomotor development had stagnated particularly between the ages of 5 and 9 months but nevertheless progressed afterwards in 2 of them The third patient had an intervening period of infantile spasms rendering difficult an adequate evaluation of the development with reference to the effect of the hydrocephalic state

Shunt operation became necessary in these 3 patients at the ages of $3^1/_2$ $3^1/$ and $2^3/_4$ years due to a new period of abnormally rapid skull growth signs of increasing intra

Table 4 *Late operated children with simple hydrocephalus i e failures of conservative management*

Age at operation (years)	Delay of operation (years)	Indication for operation	Persistent neurological defects	Mental impairment
3½	3	Recurrent rapid skull growth Decreasing cortical thickness Ataxic signs	Slight ataxia Hypotonia of the lower limbs	Scared contact problems IQ 105
3½	3³/₁₂	Continuous rapid growth of skull Signs of increased pressure	Ataxic diplegia	Emotionally unstable and infantile Normal intellectual capacity Retarded speech
2¾	2¹/₁₂	Recurrent rapid skull growth and signs of increased pressure	Slight ataxic signs	Alert and active Cocktail party syndrome

cranial pressure of the appearance of abnormal neurological features The *indications* for operation the time of dealy *from* diagnosis and the clinical characteristics at follow-up are presented in Table 4

Avoided operation

This group comprised 23 children The background of their hydrocephalus is presented in Table 1 At the final examination the clinical *findings* of the group were as follows

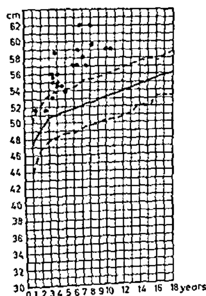


Fig 2 The head circumference at follow up examination in 23 conservatively managed children with simple hydrocephalus Normal curve according to Nellhaus (15)

Skull size and configuration The skull circumference was within normal limits in [illegible] cases the skull configuration however be[illegible] slightly hydrocephalic in these cases also Th[illegible] distribution of the deviations in the materi[illegible] was determined in cm above the 98th percentile and is shown in Fig 2 The distributio[illegible] of skull sizes in relation to pathogenetic typ[illegible] is given in Table 5 Various types of hydrocephalic skull were observed the usual form being a ballooned configuration (Fig 3) but in some only a pronounced frontal bossing or a marked general lengthening without ballooning or frontal bossing

Constitutional abnormalities Constitutional deviations of the type described in earlier reports (4) with a dysplastic body build and abnormal localization of accumulated fat were noted in 5 cases

Motor development For comparison with the non selected untreated series of Hagberg & Sjogren (1966) (4) the 23 patients were similarly divided into groups according to their degree of motor handicap As there were no severely handicapped children in the present series in contrast to that of 1966 the following three groups were chosen 1) those without any obvious motor impairment (the same criteria as 1966) 2) those with slight impair

Table 5. *Deviation in cm from normal skull circumference (>2 S.D.)*

Pathogenesis	None	0.5–1	1.5–2.0	2.5–3.0	3.5–4.0	4.5–5.0	5.5–7.0	Total
Aqueduct stenosis				1				1
Other CNS malformation	1	1		1			1	4
Basal cisternal block		1	2	2		1		6
Unknown pathogenesis	2	3	3	1	1	1	1	12
Hydrocephalus with spina bifida cystica	5	3		1	2	1	1	13
Total	8	8	5	6	3	3	3	36

ment and 3) those with moderate motor impairment. Groups 2 and 3 combined correspond to group 2 from 1966. Sixteen patients (70 %) presented no obvious motor handicap, 3 (13 %) had slight motor difficulties (2 slight ataxias, 1 clumsy child) and 4 (17 %) were moderately disabled yet moved freely. Three of these 4 had obvious though slight syndromes of cerebral palsy. The fourth with a CNS malformation exhibited marked motor retardation secondary to mental subnormality with general developmental delay.

From Table 6 it is evident that in this small material there was no correlation between the degree of increased skull *circumference* and the degree of motor impairment. Neither was





Fig. 3. Four year-old boy with spontaneously arrested hydrocephalus (basal cisternal block) together with his normal twin sister. The largest echo-index was 0.67 cm (when 3 months old) and the average cortical thickness at air encephalography 30 mm. His intellectual and motor development have always been normal. At the follow up examination his motor performances were even better than those of his twin sister. The skull circumference was 55 cm.

Table 4 *Late operated children with simple hycrocephalus, i e 'failures of conservative management*

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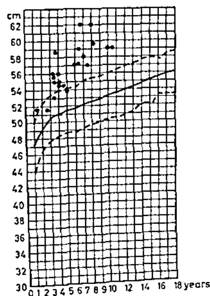


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ntellectual capacity		
Normal	Minimal subnormality	Moderately retarded
2		1
0		
[illegible]	4	
3		1
7	4	2

formances Group 2 included 2 patients with slight but obvious ataxia Group 3 included 1 ataxia 1 ataxic diplegia and 1 spastic diplegia without any ataxia Squint was present in 5 children in 2 of whom it was divergent (caused by hereditary myopia in one of them) One patient with postmeningitic hydrocephalus was deaf

Intellectual capacity All 10 children with only a slightly increased skull size (0 5–2 0 cm above normal) were without handicap and functioned on a normal intellectual level (Table 7) Among the patients with the largest skull size there was 1 with moderate mental retardation and motor impairment while 3 were intellectually normal When related to group of motor impairment some agreement was found between motor and intellectual handicap Four of the motor handicapped patients (groups 2 and 3) had mental impairment However there were also 3 motor handicapped children with a normal intellectual capacity

Behaviour deviations In 2 patients (one in group 1 one in group 2) anxiety nervousness poor self-confidence slowness and contact difficulties were apparent Two patients in group 3 had a so-called cocktail party syndrome (or chatter box syndrome) (3)

Comparison between successful expectative cases and failures

In an attempt to find some factor of value for differentiating between patients who ought to be operated on and those in whom conservative management may be chosen the patients in whom there was no obvious deleterious effect of expectative therapy (total 16)[1] were compared with the "failures Regarded as "failures were the 3 patients still living who underwent a shunt operation later and the 4 patients not operated on (motor impairment group 3) with obvious neurological or mental handicaps Of these latter 4 children 2 had malformations of the CNS which could just as well have explained the neurological picture as the hydrocephalic state in itself They were therefore excluded The group of "failures for comparison thus consisted of 5 patients

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[1] Confirmed as hydrocephalic by pneumoencephalography or ventriculography in 9 cases and by echoencephalography alone in 7 cases

Other somatic abnormalities					Intellectual capacity			
Dysplastic body build	Squint	Poor vision or optic atrophy	Epilepsy	Infantile muscular hypotonia	Normal	Minimal subnormality	Moderately retarded	Cocktail" party" speech
	[illegible][a]	1[b]	1	2	14	2		
	1			3	2	1		
1	2		2		1	1	2	2
5	5	1[b]	3	5	17	4	2	2

Table 6 *Clinical findings in relation to head circumference in simple hydrocephalus at follow up examination*

Head circum ference d viation (cm)	No of patients	Age in years at examination					Echo enc index	Thickness of cerebral mantle at air enc (cm)	Degree of motor handicap		
		1–2	2–4	4–6	6–8	8–10			No obvious handicap	Slight handicap	Moder handic
Normal	3		2	1			0 51–0 62	?[a]	2		1
0 5–2 0	10	1	4	2	1	2	0 39–0 63	2 0–4 0	10		
2 5–4 0	6		1	1	4		0 46–0 57	1 5–2 0	2	2	2
4 5–7 0	4		1		1	2	0 32–0 52	(4 5)[b]	2	1	1
Total	23	1	8	4	6	4			16	3	4

[a] No measurable value available
[b] Measurable thickness available in only one of the patients

there any correlation between the maximal echo indices during the most active stages of hydrocephalus nor the cortical thickness at air encephalography and the degree of skull circumference deviation at the final follow up examination

Taken as a simple measure of gross motor development during the first year of life was the age at which the child first walked with out support (Table 7) In motor impairment group 1 (16 cases) 12 patients walked before the age of 18 months 2 between 18 and 24 months and 2 between 2 and 4 years The motor performance for the 14 children first mentioned was found to be quite normal at follow up while the 2 late cases still displayed a slightly clumsy motor pattern in running and climbing Group 2 (3 cases) included one child who certainly walked at 16 months bı who showed a clumsy and retarded gross m tor development at follow up The two oth patients in this group first walked at 23 aı 24 months respectively and still had gait d turbances at follow up In group 3, two the 4 patients first walked between 2 and years of age while one child with spastic plegia without ataxia surprisingly enou learned to walk as early as at 15 months T fourth patient a girl with an ataxic gait fı walked when 24 months old

Neurological signs At neurological exami tion (Table 7) normal conditions were fou in motor impairment group 1, with the exc tion that 2 patients displayed a slight isola intention tremor in advanced fine motor p

Table 7 *Clinical findings in relation to the degree of motor handicap in simple hydrocephalus at foll up examination*

Degree of motor handicap	No of patients	Learned to walk without support in years			Main syndrome of cerebral palsy				
						Ataxia			
		1½	1½–2	2–4	Normal	Alone	With spastic diplegia	Spastic diplegia without ataxia	Clumsy cl
Not obvious	16	12	2	2	12	2			2
Slight	3	1	2			2			1
Moderate	4	1	1	2		1	1	1	1[c]
Total	23	14	5	4	12	5	1	1	4

[a] Convergent squint due to paresis of M rect temp
[b] Changes secondary to chorioretinitis of the right eye
[c] Clumsiness mainly secondary to imbecillity

[illegible]ntellectual capacity		
[illegible]ormal	Minimal subnormality	Moderately retarded
		1
[illegible]		
	4	
[illegible]		1
[illegible]	4	2

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Intellectual capacity All 10 children with only a slightly increased skull size (0 5–2 0 cm above normal) were without handicap and functioned on a normal intellectual level (Table 7) Among the patients with the largest skull size there was 1 with moderate mental retardation and motor impairment while 3 were intellectually normal When related to group of motor impairment some agreement was found between motor and intellectual handicap Four of the motor handicapped patients (groups 2 and 3) had mental impairment However there were also 3 motor handicapped children with a normal intellectual capacity

Behaviour deviations In 2 patients (one in group 1 one in group 2) anxiety nervousness poor self-confidence slowness and contact difficulties were apparent Two patients in group 3 had a so-called cocktail party syndrome (or chatter box syndrome) (3)

Comparison between successful expectative cases and failures

In an attempt to find some factor of value for differentiating between patients who ought to be operated on and those in whom conservative management may be chosen the patients in whom there was no obvious deleterious effect of expectative therapy (total 16)[1] were compared with the failures Regarded as failures were the 3 patients still living who underwent a shunt operation later and the 4 patients not operated on (motor impairment group 3) with obvious neurological or mental handicaps Of these latter 4 children 2 had malformations of the CNS which could just as well have explained the neurological picture as the hydrocephalic state in itself They were therefore excluded The group of failures for comparison thus consisted of 5 patients

[1] Confirmed as hydrocephalic by pneumoencephalography or ventriculography in 9 cases and by echo encephalography alone in 7 cases

Other somatic abnormalities					Intellectual capacity			
Dysplastic body build	Squint	Poor vision or optic atrophy	Epilepsy	Infantile muscular hypotonia	Normal	Minimal subnormality	Moderately retarded	Cocktail party speech
[illegible]	[illegible][a]	1[b]	1	2	14	2		
	1			3	2	1		
1	2		2		1	1	2	2
5	5	1[b]	3	5	17	4	2	2

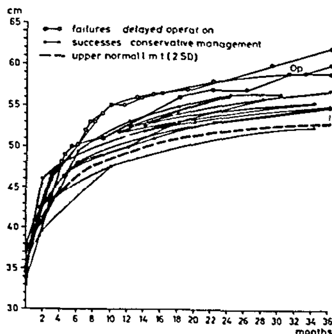


Fig 4 Comparison between the skull growth curves of patients with simple hydrocephalus subjected to shunt operation later ("failures") and those regarded as "successes" (see text). Normal upper limit according to Westropp & Barber (19)

When comparing the "failures" with the successful group of patients not subjected to operation, basal cisternal block was found to be the cause in the 5 "failures" but had also been verified in 4 of the 16 "successful" cases. *Furthermore it was probably the cause in the* majority of the 10 with an "unknown" aetiology. Aetiological analysis revealed no factors of value for differentiation. Comparison between *the growth rate of the skull in the two* groups is shown in Fig 4.

It is evident that the rapid rise of the curve did not differ during the first 4 months of life—one of them from a child in the "successful" group was in fact steeper than any of those from the "failures". After the age of 4 months however the 3 shunt patients deviated *significantly from the "successful"* cases. In the 3 shunt patients the smallest cortical thickness at air encephalography performed during the first diagnostic investigation at the paediatric clinic was 7–8 mm frontally, 15 mm parietally and 10 mm occipitally. The corresponding values in the "successful" group were 20, 20 and 20 mm respectively. In one of the non surgically treated "failures" these values were 20, 25 and 15 mm. Our series of air encephalographies are too small to allow definite conclusions to be drawn. However, in our opinion measurement of the cortical thickness alone at air encephalography is not sufficient to permit a decision concerning the choice between active and conservative management.

The psychomotor development during the first years of life had stagnated in 2 of the 3 patients operated on later. A delay was certainly noted in 9 of the 16 "successful" children. However, there was a continuous steady improvement of the developmental profile in *this group. The presence of hydrocephalic* neurological syndromes was characteristic of the "failures", being found in all five. At follow up no corresponding obvious neurological syndromes were present in any of the "successful" cases. However slight signs of dyscoordination and clumsiness were noted in 4 of the patients.

To conclude, during the *first 4 months* of life no simple reliable factor was found which could help to differentiate between patients who should undergo operative treatment and those who could be left *without a shunt*. After 4 months of age a continued marked abnormality of the skull growth accompanied by stagnation of the psychomotor develop*ment and a suspected appearance of spastic* diplegic and/or ataxic signs were found to be important to watch for with regard to the future outcome.

Age for safe operation?

The "failures" were analysed as to the latest *optimal age at which shunt operation* probably should have been performed. In 4 of the cases sufficient developmental and neurological data were available for a thorough retrospective evaluation. It was obvious that developmental stagnation and the first signs of abnormal neurologi...

manifest somewhere between 5 and 9 months of age These findings also coincided in time with pronounced continued deviation of the skull growth from the normal curve

From this small material the question thus seems to be best answered that operation probably ought to have been performed before 6 months of age to prevent the permanent neurological impairment considered to be due to the hydrocephalic state in itself

Discussion

Controlled therapeutic trials of active surgical as against conservative management in simple hydrocephalus have been performed by Lorber (7 11) in 50 cases Patients were allocated alternately by random selection for operation and expectancy respectively at the time of diagnosis of their hydrocephalus His trial in an unselected group of infants showed that th progression of the untreated disease was consistently and often rapidly unfavourable Surgical intervention became necessary in the great majority (all except 3) of those who were initially allocated to the control group As a conclusion he considers that the so-called spontaneous arrest must be rare indeed

As far as we know no purposeful attempt to select cases of simple hydrocephalus for conservative management has been made previously Merrill et al (14) mentioned one of 100 patients who was not treated surgically because the hydrocephalus was thought to be arrested spontaneously The final outcome was not mentioned however Our series of 45 cases of simple hydrocephalus in whom shunt operations were selectively avoided confirms our experience from our earlier retrospective studies (3 4) that spontaneous compensation of the hydrocephalic state sometimes occurs and that normal mental and motor developments are not too rare in spite of an increased intracranial pressure during the first period of life However our hopes of finding some simple early factors at diagnosis that might facilitate differentiation between cases where operation could be avoided with certainty and those where a shunt should be performed were not realized It is still much easier to decide to operate than to wait! Yet from our series of 16 successful cases among 27 considered suitable for conservative management it would seem that some sort of selection was possible but that the risk of failure was high during the period in question Analysis of the "failures shows that this risk of disabling neurological handicaps seems to increase significantly during the second half of the first year This finding is in good agreement with experiences from experimental hydrocephalus in young rabbits and puppies compared to the findings from cerebral biopsies in human infantile hydrocephalus (18) These studies revealed that the severity of tissue damage is to some extent proportional to the amount of myelin developed in the white matter At the same time the neuronal layers seem to remain surprisingly well preserved independent of developmental stage and even with very pronounced intracranial expansion

To conclude it seems justifiable to wait in borderline cases only during the first 5–6 months of life but no longer if the increase in skull growth then still seems uncompensated if there is an obvious developmental stagnation or regression and particularly if there is any suspicion of abnormalities of the nervous system of the type known to be associated with the chronic brain syndrome of infantile hydrocephalus On the other hand patients with a skull growth undergoing normalization with a satisfactory psychomotor development and with no abnormal neurological features probably can be spared from operation while being followed up carefully It must always be kept in mind however that blows infections intoxications etc may provoke a deterioration with a disastrously rapid increase in the intracranial pressure against closed sutures and resulting in neurological disability

To maintain one s balance between active

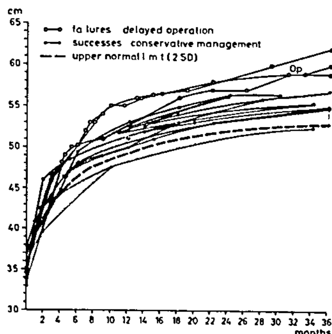


Fig 4 Comparison between the skull growth curves of patients with simple hydrocephalus subjected to shunt operation later (failures) and those regarded as successes (see text) Normal upper limit according to Westropp & Barber (19)

When comparing the failures with the successful group of patients not subjected to operation basal cisternal block was found to be the cause in the 5 failures but had also been verified in 4 of the 16 successful cases Furthermore it was probably the cause in the majority of the 10 with an unkown aetiology Aetiological analysis revealed no factors of value for differentiation Comparison between the growth rate of the skull in the two groups is shown in Fig 4

It is evident that the rapid rise of the curve did not differ during the first 4 months of life—one of them from a child in the successful group was in fact steeper than any of those from the failures After the age of 4 months, however the 3 shunt patients deviated significantly from the successful cases In the 3 shunt patients the smallest cortical thickness at air encephalography performed during the first diagnostic investigation at the paediatric clinic was 7–8 mm frontally 15 mm parietally and 10 mm occipitally The corresponding values in the successful group were 20, 20 and 20 mm, respectively In one of the non surgically treated failures these values were 20 25 and 15 mm Our series of air encephalographies are too small to allow definite conclusions to be drawn However, in our opinion measurement of the cortical thickness alone at air encephalography is not sufficient to permit a decision concerning the choice between active and conservative management

The psychomotor development during the first years of life had stagnated in 2 of the 3 patients operated on later A delay was certainly noted in 9 of the 16 successful children However, there was a continuous steady improvement of the developmental profile in this group The presence of hydrocephalic neurological syndromes was characteristic of the failures , being found in all five At follow up no corresponding obvious neurological syndromes were present in any of the successful cases However slight signs of dyscoordination and clumsiness were noted in 4 of the patients

To conclude during the first 4 months of life no simple reliable factor was found which could help to differentiate between patients who should undergo operative treatment and those who could be left without a shunt After 4 months of age a continued marked abnormality of the skull growth accompanied by stagnation of the psychomotor development and a suspected appearance of spastic diplegic and/or ataxic signs were found to be important to watch for with regard to the future outcome

Age for safe operation?

The failures were analysed as to the latest optimal age at which shunt operation probably should have been performed In 4 of the cases sufficient developmental and neurological data were available for a thorough retrospective evaluation It was obvious that developmental stagnation and the first signs of abnormal neurological conditions became

Mentally			
Normal	Slight intellectual subnormality	Moderately	Cocktail party syndrome
4	—	1	1
2	1	—	—
3	—	—	1
	—	—	—
11	1	1	2

ence at follow up Two had a markedly increased circumference (4 5–6 0 cm above normal) No correlation was found between the maximal values for their echo-indices and their skull circumference deviation at follow up (Table 8) The clinical condition at follow up examination is presented in Table 8 On account of their primary malformation the myelomeningocele conclusions could only be drawn from evaluation of the upper half of the body A normal motor development and neurological state were found in 8 children (62) slight fine motor difficulties and clumsiness in 2 children and clear but slight intention tremor in 3 Squint was present in 3 cases and 1 child had an easily controlled grand mal epilepsy

The intellectual capacity was normal in 11 children slightly subnormal in 1 child and moderately subnormal in 1 One of the latter had a hypoplastic corpus callosum in addition to his spina bifida syndrome Emotional lability was apparent in 1 boy with an IQ of 127 and 2 girls showed a characteristic cocktail party syndrome

Discussion

In this series of 14 patients in whom a shunt operation was avoided or postponed for more than 6 months no case was found which could be assessed as a real "failure The results of the mode of management in the boy operated on later offer support for our policy He was examined clinically and echo encephalographically at frequent intervals for 6 months and showed no signs of developmental stagnation or abnormal neurological features He was operated upon in a second stage with a threatening rapid skull growth Delay of the operation if possible is considered to be advantageous as the risk for shunt complications has been reported to be higher the younger the infant (11)

The favourable result in this small series confirms the opinion of Lorber (8 11) that there is a good chance of spontaneous arrest in hydrocephalus associated with spina bifida cystica particularly when the pathogenetic factor exclusively can be referred to an Arnold Chiari malformation Thus conservative management should be tried in all borderline cases However thorough and frequent follow up examinations—if possible by the same doctor—are necessary in order that cases may be picked up in the early stage of a new unexpected period of increased intracranial pressure In not so few cases a recurrence can be insidious and neurological damage and blindness may rapidly occur if not carefully watched for

GENERAL CONCLUSIONS

Experience from this series of 45 patients in whom conservative management was tried in the hope that a so-called spontaneous arrest might occur has brought us to the following conclusions firstly for the group of simple hydrocephalus and secondly for the group in which this is associated with spina bifida cystica

A Simple hydrocephalus

Spontaneous arrest or compensated balance of the hydrocephalic process occurs in some cases due to basal cisternal block and CNS malformation with the probable exclusion of aqueduct stenosis and atresia Selection of cases with a tendency to spontaneous arrest is

Table 8 *Clinical findings at follow up examination in the patients of hydrocephalus combined with spina bifida cystica*

Head circum ference deviation (cm)	No of patients	Echo enc index	Thickness in cm of cerebral mantle at air encephalography (*n*)	Main syndrome of cerebral palsy in the upper extremities			Other somatic abnormalities			
				Normal	Clumsiness	Slight but obvious ataxia	Dysplastic body build	Squint	Poor vision or optic atrophy	Epilepsy
Normal	5	0 40–0 57	2 7 (1)	4	—	1	—	1	—	—
0 5–2 0	3	0 61–0 67	? (0)	2	—	1	—	1	—	1
2 5–4 0	3	0 59–0 67	1 5–2 0 (2)	2	—	1	2		—	—
4 5–6 0	2	0 56–0 70	2 0 (1)	—	2	—	2	1	—	—
Total	13			8	2	3	4	3	—	1

surgical and conservative management in borderline cases is difficult (7 11) However we cannot agree with Lorber (9) when he states that conservative management is useless and that practically all infants with simple hydrocephalus must be operated on The important problem is rather to learn how to pick out suitable cases for conservative management It is true that we have not been too fortunate in our trials in the present series only about 70% being successful cases With the suggested approach however the risk should be diminished In view of the high risk of complications found to follow shunt procedures (13) as many cases as possible ought to be spared from operation It should be pointed out that life threatening complications of shunt procedures by no means strike only severely damaged cases of hydrocephalus but equally often primarily mild cases

While we cannot accept Lorber s negative attitude to conservative management in general (9), we agree with him that no child with simple hydrocephalus should be denied surgical treatment by reason of an extreme magnitude of the hydrocephalic state when a decision is being made regarding operation It is quite evident from our findings as well as from the experience of others that even a pronounced hydrocephalus—if not operated on too late—may be associated with a surprisingly good psychomotor development

B HYDROCEPHALUS ASSOCIATED WITH SPINA BIFIDA CYSTICA

Among the 45 children in our series there were 14 in whom the hydrocephalus was associated with spina bifida cystica

Mortality

None of the 14 patients has died

Delayed operation

One of the children was operated upon after 6 months delay from the time of diagnosis of his hydrocephalus which was present at birth Air encephalography had revealed a combination of Arnold Chiari malformation and aqueduct stenosis His hydrocephalic state seemed to have become normalized during his first month of life but a period of rapid skull growth with an increasing echo index when 6 months of age led to a shunt operation 1 month later At the final follow up his motor development at the age of 2 1/ years was slightly delayed but there were no additional neurological abnormalities taking into account the prenatal impairment from his spina bifida cystica He showed however some lack of concentration and had a slight cocktail party syndrome

Avoided operation

Among the 13 conservatively treated children there were 5 with a normal skull circumfer

entally

ormal	Slight intellectual subnormality	Moderately	Cocktail party syndrome
	—	1	1
	1	—	—
	—	—	1
	–	—	—
	1	1	2

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The favourable result in this small series confirms the opinion of Lorber (8 11) that there is a good chance of spontaneous arrest in hydrocephalus associated with spina bifida cystica particularly when the pathogenetic factor exclusively can be referred to an Arnold Chiari malformation Thus conservative management should be tried in all borderline cases However thorough and frequent follow up examinations—if possible by the same doctor—are necessary in order that cases may be picked up in the early stage of a new unexpected period of increased intracranial pressure In not so few cases a recurrence can be insidious and neurological damage and blindness may rapidly occur if not carefully watched for

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Spontaneous arrest or compensated balance of the hydrocephalic process occurs in some cases due to basal cisternal block and CNS malformation with the probable exclusion of aqueduct stenosis and atresia *Selection* of cases with a tendency to spontaneous arrest is

difficult and relapses in so called arrested cases are not unusual A trial of conservative management is justifiable during the first 5–6 months of life in slowly progressive cases of the types mentioned if the psychomotor development does not show any obvious tendency towards stagnation or regression, if no abnormal neurological features appear, and if the skull growth is undergoing normalization and the echo index is not increasing significantly

Aside from continuous follow up of an abnormal skull size with plotting on a diagram and of an enlarged echo index the following simple clinical signs should always be looked for and be given due consideration with a view to a more active approach widened sutures (particularly the lambdoid), enlarged and tense fontanelles increasing sun set phenomenon, stagnation of a previously normal psychomotor development appearence of hypotonia in the legs and feet signs suspicious of spastic diplegia in early infancy and dyssynergia and intention tremor of the hands in late infancy

B Spina bifida cystica

Hydrocephalus defined as an increased echo index is present in 90–95% of all cases with spina bifida cystica In 65–70% there is an overt hydrocephalus There is an obvious tendency towards spontaneous arrest in quite a number of cases with a slight and slowly progressive abnormal skull growth Conservative management is successful more often than in simple hydrocephalus and should always be tried during the first months of life in all borderline cases

SUMMARY

A selected series of 45 conservatively managed hydrocephalic children from a total of 179 hydrocephalic cases cared for at the Department of Paediatrics in Uppsala in 1960–1969 is presented Simple hydrocephalus was present in 31 children In 14 patients the hydrocephalus was associated with spina bifida cystica Five children in the first group died for other reasons than their hydrocephalic process *per se* in 4 (including 1 of the children who died) a shunt operation had to be performed at a later stage Among the surviving non surgically treated children with infantile hydrocephalus, 16 of 23 (70%) were in an excellent condition without any handicap From analysis of 5 thoroughly followed up failures and comparison with successful cases it could be concluded that conservative management is justifiable during the first 5–6 months of life in slowly progressing cases but no longer if the increase in skull growth then still seems uncompensated if there is an obvious developmental stagnation or if there is any suspicion of appearance of abnormal neurological features No safe criteria were found for aiding in the decision regarding operation on initial diagnosis of the hydrocephalus It was also concluded from our group with hydrocephalus associated with spina bifida cystica that conservative management more often was successful in these cases than in those with simple hydrocephalus and that a special effort should be made to avoid shunt operations in these patients

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GENERALIZED BCG TUBERCULOSIS WITH FATAL COURSE IN TWO SISTERS

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Fatal generalized BCG tuberculosis in association with BCG vaccination is extremely rare To the best of our knowledge only fourteen such cases have so far been reported (10) Although considerable progress has been made in immunology during the past 20 years the immunological deficiency in children with generalized BCG tuberculosis has not yet been completely explained Congenital agammaglobulinemia used to be considered one of the conditions conductive to extensive BCG dissemination following vaccination (1 3 4) To day however we know that children with agammaglobulinemia do develop tuberculin hypersensitivity and acquired resistance to tuberculosis following BCG vaccination (6 12) It is only during recent years that attention has been drawn to the fact that persons with deficiency of cellular immunity are exposed to the danger of vaccination with viable organisms such as BCG vaccine (11 19)

This report presents two cases (sisters) of fatal generalized BCG tuberculosis In one the illness developed after BCG vaccination while in the other it developed after BCG infection by natural route Defect of the cellular defence mechanism was established in both children

CASE REPORTS

Case 1

Marijana the family s first daughter was born October 2nd 1964 birth weight 3 000 g Both parents were non consanguineous and healthy The infant was BCG vaccinated intradermally in the left forearm when she was 4 days old She had never been in contact with a tuberculous patient The other children vaccinated with the same batch of vaccine showed no particular complications Case history showed that in the mother s family in the second generation five girl infants had died in their early months of unknown causes

Adenitis in the left axilla was established when the girl was in her fourth month At that time there was a visible 2 × 7 mm induration at the site of the BCG injection A month later the enlarged lymph node in the left axilla fistulated At the same time the other subcutaneous lymph nodes swelled and the girl developed hepatosplenomegaly In her seventh month she contracted chickenpox In her first year she suffered several times from respiratory and digestive infections Oral moniliasis was persistent Towards the end of the first year the fistula in the left axilla closed the spleen no longer surpassed the costal margin while the liver was palpable by 1 5 cm The E S R which had been 70 mm/hr at the onset of the illness was now 10 mm/hr During the first year of the illness there was hypochromic anemia (hemoglobin was 7 4 to 10 7 g/100 ml)

At the beginning of the second year more subcutaneous lymph nodes began swelling rapidly When 15 months old the girl had mumps In her seventeenth month the oral moniliasis disappeared In her third year the lymph nodes of the neck and groin developed abscesses and fistulated through the skin In her thirtieth month the patient s pulmonary radiography revealed enlarged paratracheal glands for the first time In spite of the advancing process in the lymph nodes the girl s ponderal development was not impaired Weight at the end of the third year was 15 9 kg

In the fourth year more packets of enlarged lymph nodes developed with fresh fistulate and ulcerations (Fig 1) In January 1968 the girl contracted whooping cough after which her general condition deteriorated suddenly The abdomen was enlarged due to ac



Fig. 1. Case 1. Numerous cervical ulcerations developed in the fourth year after BCG vaccination.

cumulated abdominal fluid and recurrent hepatosplenomegaly. In March maculopapular rashes appeared on the skin but disappeared after 10 days. In June the girl suffered from profuse melena with subsequent serious anaemia (hemoglobin dropped to 6.2 g/100 ml). Oral moniliasis developed again. In July the patient developed septic fever and had diarrhea. Soon thereafter generalized edema appeared. Early in September the girl refused any food and vomited intractibly. She died in a coma, completely cachectic on September 16th, 1968 at the age of three years and eleven months.

Treatment. From the onset of the illness until death the child was given, except short intervals, at least two of the following tuberculostatic drugs: Streptomycin, izoniazid, PAS, kanamycin, ethionamide and ethambuthol (Fig. 2). In the initial and final stage the child was treated for a short time with corticosteroid. Gammaglobulins were given once in preventive doses. Blood transfusions were given once in the first year and several times during the last 2 months. At the beginning of the fourth year the child was given infusions of leucocyte suspensions three times.

Immunological examination. Serum proteins varied from 5.1 to 7.5 g/100 ml. Serum gammaglobulins and immunoglobulins (IgA, IgM and IgG) were normal or increased. After whooping cough the Bordetella pertussis agglutination titer was 1/640. Three years after Polio vaccination (type 1) the neutralising antibody titer was 1/16. Plasmocytes were detected twice in the peripheral blood stream (3% and 1). In the third year there was lymphocytopenia on two occasions (2 542 and 1 496/mm³). When the child had whooping cough there was a sudden increase in lymphocytes in the peripheral blood stream (14 552/mm³), soon to be followed by lymphocytopenia, particularly during the last 2 months (984, 1 400 and 1 708/mm³). The tests of lymphocyte transformation with phytohemaglutinin (PHA) and tuberculin were negative. Mantoux tests (1 mg) were made several times, but were negative throughout (Table 1).

Bacteriological findings. Examinations were made of specimens taken from lymph node biopsies, fistula secretions and post mortem blood. The bacilli were identified at Institute for Tuberculosis in Belgrade, "Institute Pasteur" in Paris and "Statens Seruminstitut" in Copenhagen. Staining by the Ziehl-Neelsen method revealed swarms of acid fast bacilli arranged in cords. On a Lowenstein-Jensen

Table 1. *Results of immunological investigations*

Blood lymphocytes	Case 1 Terminal lympho-cytopenia	Case 2 Terminal lympho-cytopenia
TTL with		
PHA	0	0
Tuberculin	0	0
BCG		0
Candidin		0
Delayed hypersensitivity reaction to		
Tuberculin	0	0
BCG		0
Candidin		0
Varidase		0
DNCB		+
Serum immunoglobulins IgA, IgM & IgG	Normal or elevaled	Normal or elevated
Serum antibodies		
Natural		
isohemagglutinins		-
Acquired		
measles		+
salmonella enteritidis		+
pertussis	+	+
After vaccination		
polio	+	0
diphtheria		0
parotitis		+
tetanus		0
Thymus	Dysplasia	Dysplasia (?)
Peripheral lymphoid tissue	Hypoplasia	Hypoplasia
Tubercle	0	0

TTL-test = transformation of lymphocytes

medium the bacilli formed eugonic", weakly pigmented colonies, unchanged after exposure to light On Dubos' medium with Tween 80, colonies were homogeneous, and on Youmans medium granular Biochemical reactions of patient s strains were catalase + + + (70° 0), peroxidase +, nicotinic acid 0, nitrate reduction 0 or ± The strains isolated early during the illness were resistant to pyrazinamide and cycloserine, and sensitive to all other tuberculostatic drugs Later during therapy they became resistant to other drugs (Fig 2) Ten guinea pigs after inocculation with pathological material or cultures (0 01 mg, 1 mg 2 mg and 5 mg of bacterial mass) showed conversion of the tuberculin test Autopsies performed 54, 57, 73, 122, and 136 days after inocculation showed no instance of generalized infection (in two animals there were caseous inguinal lymph nodes) Tests repeated on rabbits produced the same results It was not possible on the basis of these tests to distinguish the strains of the patient from organisms of the BCG type

Biopsy findings Specimens taken from the right inguinal and axillar lymph nodes showed a blurred structure Most of the node was invaded by epithelioid cells Scanty lymph follicles were found under the intact capsule with poorly developed germinal centres

Autopsy findings Necropsy was performed 24 hours after death The girl weighed 11 kg and measured 104 cm The skin covering the enlarged subcutaneous glands was necrotic and ulcerated Greyish white nodules were visible on the internal organs (kidney suprarenal gland spleen and thyroid gland) Serofibrinous pleurisy in the left hemithorax and serofibrinous peritonitis in the abdomen with disseminated nodules on the serous membranes were found

Thymus weighed 6 g The lobular and follicular structure of thymus could not be distinguished Has sall s corpuscles were not visible (Fig 3) Visible cells diffusely distributed corresponded to thymocytes

In the lymph nodes and spleen one could see sparse and rudimental lymph follicles with poorly developed germinal centres In the other lymphoid formations situated in the tonsils small intestine appendix and the colon only diffusely distributed lymphocytes could be seen in addition to plasmocytes Ulcerations were visible in the small intestine mucosa in the area of Peyer s patches

The lymph nodes—subcutaneous mesenteric para aortal in the hilus of the spleen around the pancreas and along the trachea were considerably enlarged packed together and partially colliquated

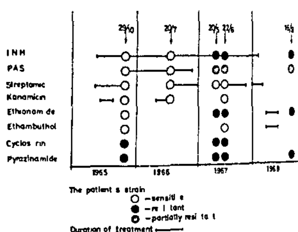


Fig 2 Case 1 Emergence of resistance to some anti tubercular drugs during treatment

Microscopic examination revealed granulomas in many organs the granulomata composed of epithelioid cells and a lesser number of lymphocytes on the periphery No Langhans giant cells were found in the granulomas No granuloma formations were found in the lungs brain thymus parathyroid glands liver or bone marrow

Case 2

Aleksandra was born on October 14th 1966 birth weight 3 150 g She did not receive BCG or other vaccines but was in frequent contact with her elder sick sister (Case 1) so that she may have possibly become infected with BCG by the natural route In her second month the girl had otitis and diarrhea Moniliasis was diagnosed at that time In her first year the girl suffered from frequent infections of the upper respiratory tract Early in her second year she had pneumonia which was cured in a fortnight with penicillin In her fifteenth month she contracted whooping cough and in her seventeenth month mumps In the patient s eighteenth month she began coughing and complained of abdominal pain At that time physical and X ray examination revealed hypoventilation in the left lower lobe Bronchoscopy was performed in June 1968 showing a granuloma just below the adit to the main bronchus on the left side After the first ablation of the granuloma respiration improved but coughing persisted The remaining part of the granuloma was removed during another bronchoscopy in August 1968 Some days after bronchoscopy the lymph nodes of the neck suddenly enlarged and following puncture a permanent fistula developed on the neck Hepatosplenomegaly and angular stomatitis were first diagnosed at that time

Early in her third year the girl s condition deteriorated she became listless and anorexic Together with the cervical lymph nodes there was swelling of pre auricular axillary and inguinal nodes More fistulas and ulcerations appeared on the skin of the neck Radiography of the lung showed a segmental shadow in the left upper lobe and heightened transparency in the left lower lobe as well as shadows indicating



Fig 3 Case 1 Thymus Undifferentiated lobular and corticomedullar structure Hasall s corpuscles absent Present cells corresponding to thymocytes (H and E × 300)

enlarged paratracheal lymph nodes (Fig 4) In December 1968 the girl contracted chickenpox the course of which was normal In January 1969 appetite improved and she started putting on weight Between March 6th and May 15th 1969 the patient was admitted to the Hopital Bretonneau in Paris where supplementary immunological and other tests were made Here radiological examination of the mediastinum did not show a shadow corresponding to the thymus While in this hospital the girl had measles

In July 1969 her general condition suddenly deteriorated The patient had fever vomited refused food and suddenly began losing weight During the last 2 3 weeks of life the girl was dyspnoeic and cyanotic She died on August 13th 1969 highly cachectic and with respiratory insufficiency at the age of two years and ten months No autopsy was performed as the parents refused permission

Treatment After the detection of acid fast bacilli in the lymph nodes the patient was given except for short intervals at least two of the following antitubercular drugs Streptomycin isoniazid rifocin kanamycin PAS rifampicine and ethambuthol The patient received blood transfusions late in her first and early in her second year

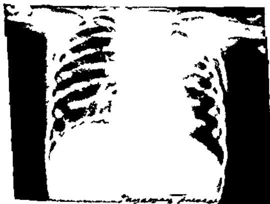


Fig 4 Case 2. Radiography of the chest in the third year of life Large number of acid fast rods (BCG) were discovered in a granuloma taken from the left main bronchus

Immunological examination Serum proteins varied between 6 2 and 7 3 g/100 ml Immunoelectrophoresis showed normal or increased values of IgA IgG and IgM The serum titer of isohamaglutinin anti B was normal (The blood group was A) Before measles the titer was <1/5 and after 1/160 After salmonella infection the antibody titer was up to 1/800 Whooping cough serum antibodies were low (1–40) After intradermal administration of mumps antigen the antibody level rose from 1/40 to 1/320 After the administration of anti DT polio vaccine (4 injections at eight day intervals) the antibody level showed practically no increase (15 days after the last dose of vaccine)

Blood leucocytes varied between 4 900 and 12 600/mm³ the absolute count of lymphocytes between 1 300 and 4 800/mm³ Plasmocytes were found (to 1 %) several times in the peripheral blood After lymphocyte stimulation with PHA lymphocyte transformation in the culture was very low (9%) while with BCG it was minimal (3 4 %) with tuberculin 0 and with candidin 0 2%

Throughout the illness all tuberculin tests

and the BCG test were negative The delayed hypersensitivity reaction against varidasis and candidin, were likewise negative However the dinitrochlorbenzene (DNCB) test was positive (Table 1)

Bacteriological findings In October 1968 when the child was 2 years old Ziehl-Neelsen stained specimens taken from the cervical lymph nodes showed numerous acid fast rods Bacteriologic investigations showed that the acid fast bacilli were indistinguishable from BCG (2 16) These strains were resistant to INH ethionamide cycloserine and pyrazin amide (sensitivity tests were significant only for resistance) Supplementary examination in Institut Pasteur showed that the isolated bacilli not only posessed BCG qualities but also that they appeared identical with the strain isolated in the elder sister (La souche parait identique a celle de Mariana)

In December 1968 a large number of acid fast rods were discovered after special fixation in a granuloma taken from the left main bronchus

Biopsy findings No lymph follicles were detected by microscopic examinataion of the rectal mucosa but only numerous plasmocytes After DT polio vaccine stimulation one could see the undifferentiated para cortical region in the homogenized structure of an inguinal lymph nodule No plasmocytes were found

Immunological examination on the parents The mother showed a weakly positive tuberculin test *In vitro* her lymphocyte transformation with PHA was low (53°) and only 3 7 with tuberculin Her serum immunoglobulin values were normal Immunological investigations the father showed no disturbance

DISCUSSION

Generalized BCG tuberculosis in both these cases was most probably due to hereditary immunological deficiency as no increased virulence was established in the isolated BCG strains (9) Immunological investigations in both cases showed a defective cellular im munity and a comparatively well preserved humoral immunity (Table 1)

We are not able to determine the immuno logical disorder responsible for development of generalized BCG tuberculosis in our pa tients, in any of the known immunological diseases with deficiency of cellular immunity (Swiss agammaglobulinemia Thymic alympho-plasia Nezelof thymic dysplasia Di George syndrome, Louis Bar syndrome Wiskott Ald rich syndrome) In our patients the illness had some similarity to Nezelof thymic dysplasia from the aspect of basic laboratory immuno-logical characteristics, but it differed in cli nical course and by the delayed hypersensitivity recation to DNCB (14, 15) Nezelof et al in 1964 (14) reported an immunologic defect characterised by delay in growth constant in fections, virus and vaccinal complications pulmonary infection with candida or pneumo-cystis carinii, and a lethal outcome some two to three months after the onset of the first symptoms of the illness In both our cases however there was a chronic evolution of the illness normal ponderal development in the first years of life, normal course of virus in fections and a clinical picture dominated by signs of BCG lesion during most of the ill ness duration In the second case there was positive delayed hypersensitivity reaction against DCNB which is not characteristic of the Nezelof type of immunological disorder In view of these differences the immunologic defect in our two patients has been defined as *hereditary partial deficiency of cellular im munity with normo or hypergammaglobu linemia*

The clinical picture and evolution of gene ralized BCG tuberculosis reported in 1953 by Meyer (13) and in 1954 by Thrap Meyer (17) were similar to those in our cases One may assume that in these cases as in those we have reported there was partial deficiency of cel lular immunity characterised by low resistance to BCG and a comparatively developed re sistance to numerous other microbic agents

A completely different immunological view must be taken of the 11 published cases of BCG infection (4 5 7 8 18) In all these cases, the clinical picture and evolution of generalized BCG infection differed from those

in our patients and the cases described by Meyer and Thrap Meyer (13 17) In these 11 cases the clinical picture was dominated by signs of deficiency of cellular immunity with or without an immunoglobulin deficiency syn drome In this group of patients the first symptoms usually appeared between the second and fourth month while death ensued between the sixth and ninth month of life There were no clinical manifestations of "BCG lesions or else they appeared in the form of an atypical ulcerative lesion at the site of the BCG injec tion or in the form of regional suppurating adenitis The sparse data available for most of these cases indicate that the immunological disorder in these 11 patients could most probably be bracketed under Swiss agamma globulinemia Thymic alymphoplasia Thymic dysplasia Nezelof This means they all would have been fatal even if not BCG vaccinated as children with this kind of immunological disorder are also less resistant to other microb c agents and usually die during the first two years

From the epidemiological aspect it is im portant that fatal generalized BCG tubercu losis can develop not only after BCG vaccina tion but also after BCG infection by natural route To the best of our knowledge our second case is the first reported one of fatal generalized BCG tuberculosis which developed after infection with BCG by the natural route The route of infection was not evident as no primary effect was found However as the first clinical signs of BCG lesion manifested themselves in the bronchial mucosa and as the first acid fast rods were detected in a specimen taken from a bronchial granuloma, one may assume that in our second case the portal of entry was in the lung Furthermore the pre sence of a major number of primary effects is not impossible as there was no development of delayed hypersensitivity to tuberculin

It is generally known that antitubercular drugs have no effect in the treatment of gene ralized BCG tuberculosis in immunodeficient children This was confirmed by our two cases One can only expect a positive effect from medicaments in patients with generalized BCG tuberculosis if their immunological restitution is successful In our first case we administered infusions of leucocyte suspensions in an at tempt to achieve immunological reactivation Although the lymphocytes were taken from tuberculin positive persons we were unable to restore tuberculin hypersensitivity in our pa tient In our second case no bone marrow transplant was given as no suitable donor was available

SUMMARY

Two sisters with fatal generalized BCG tuberculosis are reported In the first case progressive and disseminated BCG lesions emerged after BCG vaccination and in the second following BCG infection by the natural route The clinical picture of both cases was dominated by signs of "malignant scrofulosis Autopsy findings in the first case showed thymic dys plasia and hypoplasia of the peripheral lymphoid tissue In the second case biopsy speci mens of a lymph node and the rectal mucosa showed poorly developed lymphoid tissue Both cases had epithelioid granulomas in many organs but no typical tubercle structure was detected Numerous acid fast bacilli indistin guishable from BCG were isolated from the lesions

In view of clinical observations and im munological investigations the immunologic deficiency states in the two cases described has been termed as hereditary partial defi ciency of cellular immunity with normo or hypergammaglobulinemia

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr Jean Bretey (Institut Pasteur Paris) Dr Raymond Mande (Hopital Bretenneau Paris) Dr Hans Eng bæk (Statens Seruminstitut Copenhagen) Dr Ni kola Bojanic (Military Medical Academy Belgrade) and Božena Zecević (Mother and Child Health In stitute Belgrade) for their assistance in the investiga tion of the presented patients

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FIBRINOLYSIS IN PRE TERM INFANTS AND IN INFANTS SMALL FOR GESTATIONAL AGE

H EKELUND and O FINNSTRÖM

From the Coagulation Laboratory and the Department of Paediatrics University of Lund General Hospital Malmo Department of Paediatrics University Hospital Umeå Sweden

Ciulla & Luraschi 1954 (10) were probably the first to report on the fibrinolytic activity in "prematures" The activity in umbilical cord blood from 9 newborns delivered in the eighth month of pregnancy was found to be high The results of later investigators have been dis cordant demonstrating both higher and lower fibrinolytic activity in "prematures" than in full term infants or adults

Most authors have found the plasminogen levels in "prematures" to be lower than in full term infants but opinions have differed con cerning the inhibitors of fibrinolysis which have been reported as higher or lower than in full term infants

This paper describes the fibrinolytic activity and the development of the various factors of the fibrinolytic system in infants of various gestational ages and in infants small for gesta tional age

CLINICAL MATERIAL

The material consisted of 162 infants who were examined at the Departments of Paediatrics in Malmo and Umeå between April 1968 and April 19 0 The material was not a random collection but consisted of infants in whom the umbilical vessels had been catheterised for sampling of blood for diagnostic therapeutic or prophylactic purposes

Each infant was classified according to its g sta tional age and birth weight (Fig 1) *Gestational age* in weeks was calculated from the first day of the mother s last menstrual period defining weeks in accordance with the principles for constructing the Swedish standard curves for the relation between weight and gestational age (14 40) i e 40th week = days 274–280 etc

Pre term gestational age less than 39 weeks (less than 267 days)

Term gestational age 39 to 4_ weeks (267–294 days)

SGA = small for gestational age infant with birth weight below the 10th percentile according to Swe dish standard curves

AGA = appropriate for gestational age infant with birth weight between the 10th and 90th percentile

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The Swedish standard curves do not cover age ranges below 33 weeks but as could be judg d from a comparable standard curve (43) *the infants below* 33 weeks were all AGA and were consequently clas sified as such

With respect to gestational age the material was divided into four groups 1) less than 33 weeks 2) 33–35 weeks 3) 36–38 weeks 4) 39–42 weeks

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1 The asymptomatic group consisted of 84 infants who had Apgar scores of 7–10 (5) at 10 min and who were afterwards apparently healthy and without any of the symptoms described below 21 of these infants had Apgar scores of <7 at 1 and/or 5 min but ≥ 7 at 10 min

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Table 1 *The total material (162 infants) grouped according to degree of maturity complications of pregnancy and/or delivery and the condition of the infant*
N normal C complicated SGA AGA LGA small appropriate large for gestational age

									Complications of				
									Pregnancy			Infant	
	Pregnancy	Delivery	Pre term SGA	Pre term AGA	Pre term LGA	Term SGA	Total		Internal	Obstetrical	Delivery	Alive	Dead
Asymptomatic group	N	N	8	6	—	16	30		—	—	—	All	—
	C	N	5	7	—	3	15		7	8	—		—
	N	C	1	6	—	8	15		—	—	15		—
	C	C	5	16	1	2	24		15	10	27		—
Total			19	35	1	29	84		22	18	42		
Symptomatic group	N	N	5	15	—	2	22		—	—	—	15	7
	C	N	—	12	—	3	15		7	10	—	8	7
	N	C	—	13	—	2	15		—	—	16	9	6
	C	C	1	21	1	3	26		14	16	28	17	9
Total			6	61	1	10	78		21	26	44	49	29

1 Fibrinolytic activity of plasma and resuspended euglobulin precipitate fibrin plate method (4)

2 Fibrin fibrinogen degradation products (FDP) in serum and serum from blood collected in a tube with an antifibrinolytic agent ε aminocaproic acid (EACA) immunochemical method (29) Since the difference between the amount of FDP *in serum and in serum EACA reflects the plasminogen activator* activity we used this difference between pairs of such samples for measuring the fibrinolytic activity (= FDP in paired sera")

3 Plasminog n immunochemical method (12 18)

4 α macroglobulin esterolytic method (17)

5 Antiplasmin activity (progressive antiplasmin") fibrin plat method (13)

6 Inhibitors of plasminogen activation clot method (33)

7 Fibrinogen spectrophotometric method (31) The volume used was half of that employed for adults

8 The haematocrit was determined with a micro capillary centrifuge (Cellokrit AB Lars Ljungb rg & Co Stockholm)

Statist cal methods

Significance of differences between the factors in various gestational age groups were studied with the one way analysis of variance Correlations between the various factors and gestational week were studied with simple linear regression analys s The signifi cance of differences between means was estimated w th Student s *t* test

RESULTS

The aim of this investigation was to study the relation between fibrinolysis on one hand and gestational age and intra uterine growth rate on the other

The material was heterogenous in the fol lowing respects which were taken into account in the evaluation of the results (*a*) time of sampling after parturition (*b*) arterial versus venous blood samples (*c*) uncomplicated ver sus complicated pregnancy and/or delivery (*d*) asymptomatic versus symptomatic groups of infants

The possible influence of these factors on the fibrinolytic activity will be dealt with se parately below No such influence could be demonstrated on plasminogen α_2-macroglobu lin antiplasmin and fibrinogen But since the findings in the asymptomatic and symptomatic groups are of clinical interest they are pre sented separately in all the figures

Fibrinolytic activity on fibrin plates was studied in 54 infants altogether 35 in the asymptomatic group (gestational ages 30–42 weeks) at intervals between 10 min and 55 hours (median 2 hours) after parturition and in 19 in the symptomatic group (*gestational ages 25–40* weeks) at intervals between 40 min and 16 ¹/₂ hours (median 1 hour 20 min) after parturition The activity in the plasma and in the euglobulin fraction was studied on un

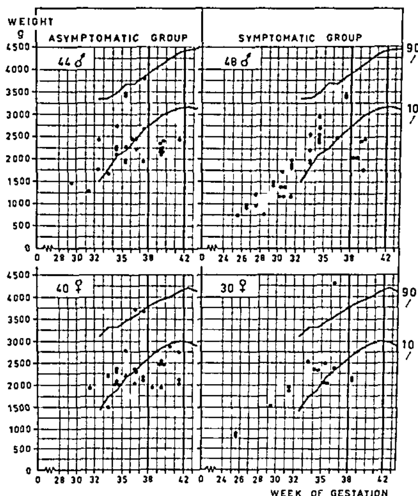


Fig 1 Distribution of the material (167 infants) according to gestational age birth weight and condition of the infant Swedish standard curves for intrauterine growth (40)

3) unspecified respiratory symptoms or other respiratory diagnoses e g aspiration syndrome or 4) intracranial haemorrhage None of the infants had neonatal infections or isoimmunizations Four infants were shown to have congenital heart disease which was part of a trisomia E syndrome in 2 of them

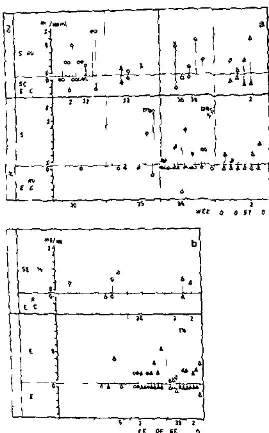
The infants were also separated according to the course of pregnancy and parturition (Table 1) The complications of pregnancy were both internal (e g diabetes toxaemia hepatosis) and obstetrical (e g premature separation of the placenta placenta praevia other bleedings premature rupture of the membranes) Complications of delivery consisted of malpresentations instrumental delivery (forceps vacuum extractions) and caesarean section With respect to these complications the asymptomatic and symptomatic groups could each be divided into four subgroups The distribution of the infants within each of these four subgroups was roughly the same in the asymptomatic and in the symptomatic group

Blood sampling

The blood samples were obtained with indwelling plastic catheters (feeding tube French size Nos 8 5 or 3 ¹/₂) in the umbilical vessels In the asymptomatic group 37 samples were obtained from an umbilical artery and 47 from the umbilical vein The corresponding numbers in the symptomatic group were 50 and 28 The time of sampling after parturition varied according to the indications for catheterisation Only 12 infants were examined within the first half hour of parturition The largest number of samples (76 infants) was obtained within 1–2 hours of parturition In the asymptomatic group the interval varied between 10 min and 68 hours with a median of 2 hours in the symptomatic group between 20 min and 138 hours with a median of 1 hour 40 min In the Umeå material blood was drawn with an ordinary glass syringe and collected only for preparation of serum In the Malmo material blood was drawn with a disposable plastic syringe and collected in siliconized glass tubes and disposable plastic tubes in order to perform if possible all the analyses mentioned below The condition of the infant and technical difficulties sometimes limited the amount of blood obtained and it was not always possible to perform the complete set of determinations

Laboratory procedures

Citrated plasma and serum were prepared in the way described previously (30 33) Serum was frozen immediately after centrifugation of the blood and stored at −20 until analysis The following determinations were made



infants in the gestational age groups 33–35 weeks and 36–38 weeks. But the mean for the group of term SGA infants (35.3%) differed significantly from what was previously found (12) for healthy full term newborns (43.4%), $t=2.75$, $p<0.01$. This difference was caused by a low mean for the symptomatic group of the term SGA infants (29.3%), $t=2.73$, $p<0.01$. No such difference could be demonstrated for the mean in the asymptomatic group (37.7%). However, the difference between the means for the asymptomatic and symptomatic groups of the term SGA infants was not significant.

A simple linear regression analysis was performed on the entire material of 143 values

Fig. 3. Estimation of plasminogen activator activity as the difference in determinations of FDP in serum and serum EACA FDP in paired sera". Relation to *gestational age and condition of the infant.* (*a*) 57 infants studied within the first 2 hours of life (31 in asymptomatic group, 26 in symptomatic group). (*b*) 34 infants studied between 2–12 hours of life (23 in asymptomatic group, 11 in symptomatic group). Symbols as in Fig. 2.

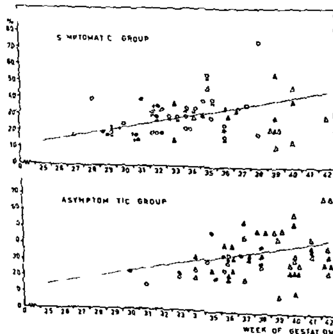


Fig. 4. Determinations of plasminogen in relation to gestational age in 143 infants (72 in asymptomatic group, 71 in symptomatic group). The regression line is obtained from the equation $y=1.75x-28.91$. There is a significant correlation between plasminogen and gestational age ($r=+0.43$, $p<0.01$). Symbols as in Fig. 2.

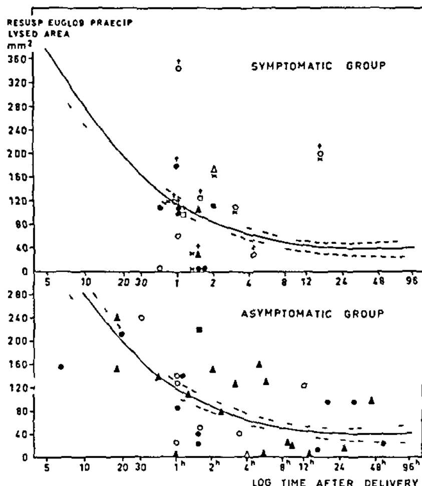


Fig 2 Fibrinolytic activity of resuspended euglobulin precipitate on unheated fibrin plates in 54 infants 35 in asymptomatic group (gestational ages 30–42 weeks) 19 in symptomatic group (gestational ages 25–40 weeks) Correlation between lysed area in mm² and the time after delivery The values are plotted against a curve obtained from a study of healthy full term newborns (12) Unbroken line mean activity Broken lines 95 confidence intervals ○ AGA infants △ SGA infants □ LGA infants Open symbols arterial blood Filled symbols venous blood × IRDS † dead

heated plates The activity in plasma was somewhat lower but closely followed that of euglobulin fraction Therefore suffice it here to give only the activity in the euglobulin fraction which in Fig 2 is plotted against the mean activity in healthy full term newborns (12) The activity in the asymptomatic and in the symptomatic group closely follows the curve for that for full term infants with the same spread around the mean A relatively low value (158 mm) at 10 min was obtained for venous blood from one infant (gestational age 30 weeks) in the asymptomatic group while the highest activity (344 mm) was found for arterial blood in a deeply asphyxiated 1-hour old infant (gestational age 31 weeks) No activity could be demonstrated in 4 infants (1 to 5 1/2 hour old) in the asymptomatic group and in 3 ones (40 min to 1 1/2 hour old) in the symptomatic group

The plasminogen activator activity was judged also from determination of the 'FDP in paired sera in 91 infants altogether within the first 12 hours of life In the asymptomatic group such activity was found in 23 of 31 infants within the first 2 hours of delivery (Fig 3 *a*) and in 19 of 23 infants between 2-12 hours after parturition (Fig 3 *b*) In the symptomatic group activity was found in 20 of 26 infants within 2 hours and in 9 of 11 between 2 and 12 hours No differences could be demonstrated with respect to gestational age or between the asymptomatic and symptomatic groups

Plasminogen (Table 2 Fig 4) was studied in a total of 143 infants 72 in the asymptomatic group (30–42 weeks) and in 71 in the symptomatic group (25–42 weeks) One way analysis of variance showed significant difference between the mean values in the various gestational age groups The F quotient for the whole group was 7 56 ($p < 0\ 01$)

No significant differences were found between the means for the AGA and the SGA

No significant differences were found between the means for the AGA and SGA infants in the gestational age groups 33–35 weeks and 36–38 weeks The mean for the group of term SGA infants (174 8%) did not differ significantly from what was previously found (12) for healthy full term newborns (169 7%) A simple linear regression analysis was performed on the total material of 67 values together with 138 values from term AGA infants (12) 205 infants in all The regression line was obtained from the equation $y = 8\,41 \quad x - 171\,29$ ($r = +0\,59 \quad p < 0\,01$)

Antiplasmin (Table 2 Fig 6) was measured in a total of 47 cases 26 in the asymptomatic group and 21 in the symptomatic group The mean values found for the various gestational age groups were at adult level without significant differences between the groups (F quotient for the total group 0 99 $p > 0\,05$)

Inhibitors of plasminogen activation (Table 2 Fig 7) could be measured in 12 cases with

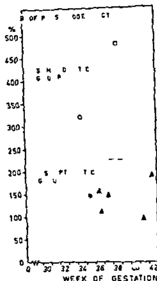


Fig 7 Determinations of inhibitors of plasminogen activation in relation to gestational age in 12 infants (9 in asymptomatic group 3 in symptomatic group) Statistical analysis not warranted Symbols as in Fig 2

gestational ages 32–42 weeks The number was too small to warrant statistical analysis Nine infants belonged to the asymptomatic group and contained values between 95 and 190 % i e the same level as in full term healthy newborns (12) There were no noticeable differences with gestational age All three values in the symptomatic group were higher than 250 % The mothers had not received EACA which since it passes over to the infant would interfere with the determination

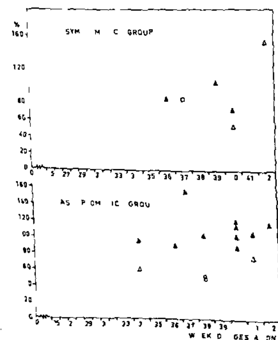


Fig 6 Determinations of antiplasmin in relation to gestational age in 47 infants (26 in asymptomatic group 21 in symptomatic group) No significant changes with gestational age Symbols as in Fig 2.

Fibrinogen (Table 2 Fig 8) was assayed in a total of 40 infants 27 in the asymptomatic group (31–42 weeks) and 13 in the symptomatic group (30–40 weeks) In order to avoid differences due to changes with time after parturition (12) only samples obtained within the first 24 hours of life were included In the gestational age group of less than 33 weeks only 5 values were obtained and the range of variation was wide mean and S D $0\,29 \pm 0\,18$ g/100 ml In the group of term SGA infants the corresponding values were $0\,21 \pm 0\,05$ g/100 ml No significant differences were found between gestational age groups (F-quotient 0 96 $p > 0\,05$) Neither was any signifi-

Table 2 *Results of determinations of plasminogen the inhibitors of fibrinolysis and fibrinogen grouped according to gestational age and birth weight Comparison with the values from a previously published study of healthy full term newborns (12)*

		Pre term							Term SGA	F quotient	p	Healthy full term newborns
		AGA	AGA	SGA	Total	AGA	SGA	Total				
Plasminogen	Mean	24 0	30 7	29 0	30 3	33 8	35 5	34 7	35 3	7 56	<0 01	43 4
	S D	8 4	9 9	10 0	9 8	12 0	10 7	11 1	14 9			16 0
	n	34	26	8	34	22	16	40 [a]	35			162
α_2 macro-globulin	Mean	86 2	122 9	93 5	114 5	147 5	128 5	138 0	174 8	14 56	<0 01	169 7
	S D	33 9	38 5	8 1	35 1	24 0	48 0	37 4	45 1			37 3
	n	14	10	4	14	11	11	23 [a]	16			161
Antiplasmin	Mean	91 3	83 5	77 5	81 5	81 1	98 2	87 2	99 8	0 99	>0 05	—
	S D	21 1	17 5	—	17 4	22 0	29 6	24 8	26 9			—
	n	13	4	2	6	10	6	17 [a]	11			—
Inhib of plasminogen activ	Mean	197 0	138 5	320 0	199 0	110 0	136 3	199 8	142 5	—	—	183 7
	S D	—	—	—	—	—	23 7	157 9	—			76 ᵓ
	n	2	2	1	3	1	3	5 [a]	2			1 8
Fibrinogen g/100 ml	Mean	0 29	0 27	0 18	0 26	0 23	0 19	0 21	0 21	2 24	>0 05	0 ᵓ6
	S D	0 18	0 09	—	0 09	0 03	0 07	0 06	0 05			0 06
	n	5	8	1	9	7	6	14 [a]	12			130
Weeks of gestation		<33	33–35			36–38			39–42			

[a] Includes one or two LGA infant(s)

and 141 values from a previously published normal series (12), 284 infants in all The regression line was obtained from the equation $y = 1.75\ x - 28.91$ ($r = +0.43$, $p < 0.01$)

α₂-macroglobulin (Table 2 Fig 5) was measured in a total of 67 cases, 41 in the asymptomatic group (30–42 weeks) and 26 in the symptomatic group (27–42 weeks) One way analysis of variance showed significant differences between the means in the various gestational age groups The F quotient for the total group was 14 62 ($p < 0.01$)

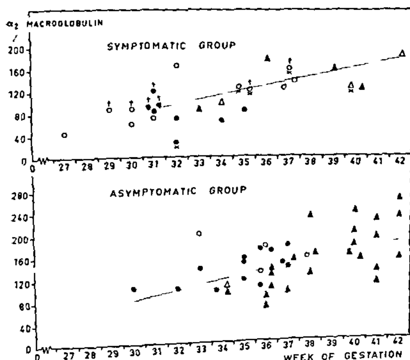


Fig 5 Determinations of α_2-macroglobulin in relation to gestational age in 67 infants (41 in asymptomatic group 26 in symptomatic group) The regression line is obtained from the equation $y = 8.41\ x - 171.29$ There is a significant correlation between α_2-macroglobulin and gestational age ($r = +0.59$ $p < 0.01$) Symbols as in Fig 2

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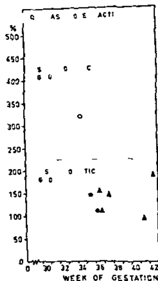


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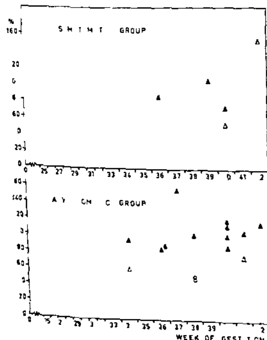


Fig 6 Determinations of antiplasmin in relation to gestational age in 47 infants (26 in asymptomatic group 21 in symptomatic group) No significant changes with gestational age Symbols as in Fig 2

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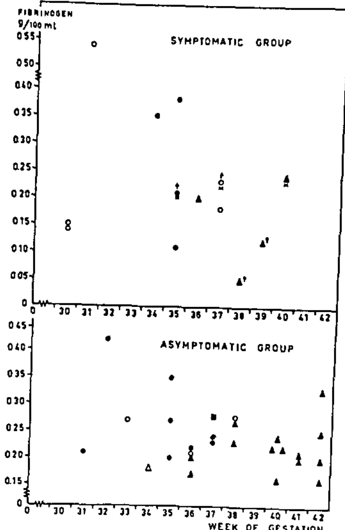


Fig 8 Determinations of fibrinogen in relation to gestational age in 40 infants (27 in asymptomatic group 13 in symptomatic group) No significant changes with gestational age Symbols as in Fig 2

cant difference found when the values were compared with those of a group of 130 healthy full term newborns (12) where samples were obtained 0–24 hours after parturition (mean and S D 0 26±0 06 g/100 ml) The mean and S D for the total material regardless of gestational age was 0 23±0 09 g/100 ml According to the method used the fibrinogen values are calculated for a haematocrit of 40% In this material the haematocrit was estimated in only 18 infants in 14 together with fibrinogen The ranges was 40–69% with a mean of 55 3% Using this value for calculating the fibrinogen values the differences were only 0 02–0 03 g/100 ml which is of no practical importance

DISCUSSION

162 pre-term infants and infants small for gestational age were studied regarding fibrinolytic activity and the development of the various factors of the fibrinolytic system related to gestational age The material was divided into an asymptomatic group and a symptomatic group comprising infants with respiratory difficulties and intracranial haemorrhages

The fibrinolytic activity as measured on fibrin plates or by determination of FDP in paired sera ', was in most cases the same as in full term infants and independent of gestational age On the whole, there were no remarkable differences betwen the asymptomatic and symptomatic groups However, two deeply asphyxiated infants showed a high fibrinolytic activity at 1 and 16 hours of life, respectively

In previous investigations of 'prematures both high and low fibrinolytic activity has been reported Studying plasma clot lysis Ciulla & Luraschi (10) found high fibrinolysis in prematures , which they ascribed to foetal anoxia With a semiquantitative immunological method Karitzky et al (22) found the fibrinolytic activity in the first 2 hours of life of both healthy prematures and prematures with IRDS (birth weights 870–2 500 g) to be higher than in adults Forgacs et al (15) on the other hand who used a clot lysis method found the same activity in a group of very low weight infants (750–1 500 g) as in full term infants Nielsen (28) who like us used the fibrin plate method found somewhat lower values in healthy premature infants than in full term infants Here the blood was obtained via a catheter in the umbilical vein within 20 min of parturition Nielsen s results agree with those of Arustowicz (6) and Markarian et al (26) who found longer euglobulin clot lysis times in prematures than in full term infants Phillips & Skrodelis (34) and Breton et al (8) demonstrated a varying degree of plasma clot lysis in about one third of their cases Ambrus et al (2) found only traces of plasminogen activator activity in 25° of a group of healthy prematures

The differences in the results can probably be explained by differences in sampling tech nique in laboratory methods and in the state of health of the prematures selected More over as pointed out previously (12) the plas minogen activator activity varies widely with the interval between sampling and analysis and with the temperature of the sample just before analysis Our study performed with standardised sampling and laboratory methods showed that pre term infants and infants small for gestational age produced the same fibrinolytic activity as healthy full term in fants

Plasminogen was determined with an im munochemical method in which blood is col lected in the presence of EACA in order to prevent plasminogen activation *in vitro* Dur ing the period of gestational age studied (25–42 weeks) we demonstrated a significant in crease of the values from levels equal to those in human foetuses (13) up to levels bordering those in full term infants As to the pre term infants we could not find any significant dif ferences between AGA and SGA infants or betwen healthy and sick infants But the plas minogen level in the small group of sick term SGA infants was significantly lower than that in healthy full term newborns This difference must be evaluated with caution because the material was small No clinical significance of this finding could be demonstrated

Earlier investigations have shown that the plasminogen level is lower in "prematures than in full term newborns (1 16 24 25 28 34 35 38 39) Phillips & Skrodelis (34) and Samart zis & Cook (38) found the levels to rise with birth weight On the other hand Karitzky et al (?1) were not able to show any correlation between plasminogen levels and birth weights in a group of apparently healthy prematures Quie & Wannamaker (35) found the plasmino gen levels of "prematures to be about $^1/_4$ and of the full term infant $^1/$ of the adult level Ambrus et al (2 3) were not able to demonstrate any plasminogen at all until shortly before term apparently because the levels were below the sensitivity of the method used Our investigation showed that plasminogen values between 25–50% of adult values are normal for pre term infants and need not be interpreted as a sign of a defect in the fibrinolytic system The concentrations are sufficient to allow production of a high fibrinolytic activity

Concerning *the inhibitors of fibrinolysis* in pre term infants most earlier investigators have reported on the total antiplasmin activity of serum As with full term infants no unani mity has been achieved regarding the levels Some investigators have reported values higher than or at the level of full term infants (2 16 24 38 39) whereas others have reported lower values (34 35) According to Phillips & Skro delis (34) the inhibitor levels tend to rise with birth weight In our study the level of *pro gressive antiplasmin* activity was the same as for full term infants throughout the gestational period studied We found a significant in crease of α_2-macroglobulin from the 27th to the 42nd week of gestation without any sig nificant differences between AGA and SGA infants or between healthy and sick infants This result is in full agreement with that of Ludwig (25) who demonstrated a significant increase of α macroglobulin from the 32nd to the 45th week of gestation but not with that of Mendenhall (27) who found no in crease with increasing "foetal size

No reports are available on *the inhibitors of plasminogen activation* in infants of low birth weight We found the levels studied from the 32nd to the 42nd week (asymptomatic group) to be equal to those in healthy full term in fants The three values in the symptomatic group were all above this level It is difficult to draw any conclusions from these few ob servations but raised levels of inhibitors of plasminogen activation have been demon strated in various pathological conditions in adults (19)

The *fibrinogen* content showed no signifi cant changes with gestational age the levels being the same as in full term infants There

was, however a wide spread, especially in the lowest gestational age group but without any significant correlation to the condition of the infant Our results agree with those obtained in some earlier investigations, where even a tendency to higher values were noted in the early pre term infant as compared with full-term infants (9 26 28 34 36, 37, 42) Levels lower than those in the full term infant have been found by Forgacs et al (15) and Arustowicz (6) Karitzky et al (23) demonstrated low levels at birth but a normal adult level within the first 12 hours of life They found no correlation between fibrinogen content and birth weight

In conclusion, this study has shown that blood from pre term infants contains plasminogen which rises significantly with gestational age and a definite plasminogen activator activity α macroglobulin also rises significantly with gestational age Progressive antiplasmin ' inhibitors of plasminogen activation and fibrinogen show the same levels as in full term newborns and adults throughout the gestational period studied Despite a low content of plasminogen the pre term infant possesses a considerable fibrinolytic capacity The production of fibrinolytic activity may possibly be favoured by a low content of α macroglobulin There were no noticeable differences between AGA and SGA infants of comparable gestational ages Thus a reduced intra uterine growth rate does not seem to influence the development of the factors in the fibrinolytic system

The investigation together with a previous study (13) which showed that human foetuses have a considerable fibrinolytic activity despite low plasminogen values, argues against a primary deficiency of the fibrinolytic system in pre term infants and infants small for gestational age It does not support the hypothesis of such a deficiency being a factor in the pathogenesis of the idiopathic respiratory distress syndrome (3) or disseminated intravascular coagulation (7) in the newborn

SUMMARY

The fibrinolytic system was studied in the blood from 162 pre term infants and infants small for gestational age The material was divided into an asymptomatic and a symptomatic group, which were approximately of the same size

Plasminogen was demonstrated in the earliest pre term infants in an amount equal to about 25% of that in adults The content increased significantly with gestational age up to term level (approx 50% of adult level) Plasminogen activator activity was found to b of the same strength as in the full term infant throughout The α macroglobulin level increased significantly from a mean of about 86% of adult content in the earliest pre term infants up to term level (approx 170%) while progressive antiplasmin ', inhibitors of plasminogen activation and fibrinogen were at term level during the whole period studied No differences were found for the various factors between infants appropriate for gestational age and small for gestational age or between the asymptomatic and symptomatic group of infants

It is concluded that the fibrinolytic system is functionally well developed already from early pre term period These findings argue against the hypothesis of a primary deficiency of the fibrinolytic system being a factor in the pathogenesis of the idiopathic respiratory distress syndrome or disseminated intravascular coagulation in the newborn

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PROGNOSIS AND LUNG FUNCTION IN CHILDREN WITH BRONCHIAL ASTHMA AND RECURRENT PNEUMONIA

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Bronchial asthma in children is often complicated with recurrent pneumonia and/or atelectasis (2 5 11) A previous clinical study of 14 children with this symptom complex showed the frequency of asthmatic attacks to decrease with increasing age (5) Investigation of the overall lung function in these 14 children showed subclinical bronchial obstruction overinflation of the lungs and abnormal unevenness of ventilation (6) An investigation of the regional lung function suggested regional impairment of lung function due to sequelae after the recurrent pneumonia The present study concerns a reinvestigation of the 14 children performed 4 years after the previous studies The purpose was to find out the further course of the disease and with the help of renewed clinical examinations and lung function studies try to estimate the prognosis in children with bronchial asthma and recurrent pneumonia.

METHODS

Spirometry and N_2-wash-out technique

Vital capacity (VC) forced expiratory volume in 1 second (FEV) and maximal voluntary ventilation (MVV) were measured as described previously (7) with the patient sitting Functional residual capacity (FRC) lung clearance index (LCI) wash-out volume and the index of alveolar ventilation (IAV) (7 12) were studied with a N_2-elimination procedure All volumes were expressed as a percentage of predicted normal values FEV was also expressed as a percentage of VC (FEV%) and FRC as a percentage of total lung capacity The normal ranges of these parameters are derived from findings in healthy schoolchildren examined at this laboratory (7)

Radiospirometry

^{133}Xe radiospirometry was performed as described previously with the patient in the supine position (8 13) The distribution of *perfusion* was estimated during breathholding after an intravenous injection of about 0 2 mCi of 133Xe The distribution of *ventilation* was measured after three normal inhalations of ^{133}Xe in a closed spirometer system containing 0 5 mCi ^{133}Xe per litre The distribution of lung *volume* (FRC) was estimated after rebreathing in this closed spirometer system until the ^{133}Xe-concentration was the same throughout in the lung spirometer system Regional *vital capacity* (VC) was measured during maximal inhalation and exhalation when this equilibrium was reached

The activity in one basal and one apical field of each lung was measured by one ventral and one opposing dorsal scintillation detector covering each field The impulses from the ventral and dorsal detector over each field were added and registered in one of four channels After subtraction of the preceding background activity the activities registered over the four fields were added and the activities registered for *each lung* were expressed as a percentage of the sum as in bronchospirometry The activity registered for each *basal field* was also expressed as the percentage of the activities registered in the two basal fields The activity registered for *each apical* field was expressed in a corresponding way

With a complete radiospirometric investigation the most critical organ—the trachea—receives about 0 1 rad (13)

In 13 of the asthmatic children duplicate deter

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Key words Fibrinolysis plasminogen inhibitors of fibrinolysis fibrin/fibrinogen degradation products, fibrinogen newborn pre term small for gestational age

Table 4 No of children with abnormal findings on physical examination and at investigations of lung volumes ventilatory capacity or ventilatory efficiency investigated 1966 and 1970

Physical examination		VC	FEV	MVV	FRC	FRC/TLC	Wash-out vol	LCI	IAV
1966	3	0	6	2	9	8	6	1	5
1970	0	0	4	2	5	6	8	0	4

corresponding values 4 years ago which indicates decreasing overinflation of the lungs The mean MVV was significantly abnormal whereas the mean MVV obtained 4 years earlier was normal The change of the mean MVV between these two investigations was however not significant

What concerns the number of children with abnormal lung function there were only slight differences between the investigations 1966 and 1970 (Table 4)

In Fig 1 the individual values found for FEV% FRC and IAV are compared with the values found in 1966 It is evident from the figure that there is a tendency to improvement of FEV% and FRC between the two investigations and that there are fewer extremely abnormal values 1970 than 1966

Regional lung function

Fig 2 gives the results as the differences between the observed individual values and the predicted normal values for the total right lung For comparison the values found in 1966 are also given Fig 3 gives the results for the two fields of the right lung Predicted normal value is the mean value for the right lung of 12 children without cardiopulmonary disease (8) In these figures values above the upper limit of the normal range (M+2 S D for the 12 controls) should be regarded as indicating a relative decrease of the function in the left lung It is evident from Fig 2 that there is no systematic change between the two investigations and that permanently decreased function of the right lung was found in only one child

Table 5 gives the means and the variation of the parameters for the total right lung and its basal and apical fields The mean values did not differ significantly from normal The S D values of some variables were significantly larger than in the control series (F

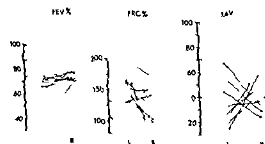


Fig 1 FEV% FRC (as a percentage of predicted normal) and IAV in 14 asthmatic children investigated 1966 (I) and 1970 (II) The points denote the individual values. For the children with abnormal values on one or both investigations the values are connected with a line N denotes the normal range

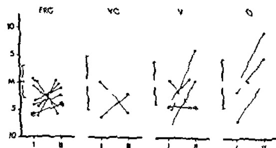


Fig 2 Regional function of the right total lung in 14 asthmatic children investigated 1966 (I) and 1970 (II) FRC = regional lung volume VC = regional vital capacity V = regional ventilation Q = regional perfusion The points denote the individual values expressed as the difference from normal mean (M) for the right lung O denotes a patient with permanently decreased FRC and V of the right lung For the children with abnormal values on one or both investigations the values are connected with a line N denotes the normal range

Table 1 *Age (years) and height (cm) in 14 children with bronchial asthma and recurrent pneumonia*

	Onset of asthma (age)	Follow up 1966		Present follow up 1970	
		age	height	age	height
Mean	3 5	9 1	133	13 3	156
Range	0 3–8 0	6 1–12 7	116–162	9 9–16 9	146–168

Table 2 *Severity of asthma in 14 children with bronchial asthma and recurrent pneumonia*

No of children with	Worst year	Follow up year 1966	Follow up year 1970
No attacks of dyspnoea	0	0	10
1–5 attacks of dyspnoea	1	10	2
>5 attacks of dyspnoea	13	4	2

minations were made of perfusion ($\dot{Q}$) on one and the same occasion

Standard statistical methods were used (14 15)

MATERIAL

Clinical data on the 14 children with bronchial asthma and recurrent pneumonia are given in Tables 1 and 2 A detailed account of the clinical findings 4 years earlier (1966) was given in a previous publication (5) The children had had severe asthma starting in early age They had had 2–11 attacks of X ray verified pneumonia (mean 5 attacks) The pneumonia had involved most frequently the right lung (ratio 72 43) with a preponderance to the middle lobe

RESULTS

Clinical findings

During the intermediate four years (1966–1970) the severity of the asthmatic symptoms had decreased as judged by story (Table 2) No child had episodes of long lasting cough or morning cough Only one child had had a new attack of X ray verified pneumonia (in the left lower lobe)

As in previous investigations all children were investigated when they were at their best They had no infection of the respiratory tract and none of them was receiving any drugs

Auscultatory findings were normal as were ESR, electrocardiogram and chest X ray Height and weight were within normal limits in all children

Overall lung function

Table 3 shows the results of measurements of lung volumes, ventilatory capacity and ventilatory efficiency for the children as a group For comparison corresponding values from the investigation 1966 and the significance levels of the differences between the obtained means and the mean values of healthy childen are also given From the table it is evident that the children as a group had still bronchial obstruction (significantly decreased FEV%) overinflation of the lungs (significantly increased FRC and FRC/TLC quotients) and abnormal uneven ventilation (significantly increased wash out volume and decreased IAV) The mean FEV% value was better (higher) than corresponding mean value four years ago This increase was however, not significant The mean FRC/TLC quotient and the mean FRC were significantly lower than

Table 3 *Lung volumes ventilatory capacity and ventilatory efficiency in 14 children with bronchial asthma investigated 1966 and 1970*

		VC	FEV	MVV	FRC	FRC/TLC	Wash out vol	LCI	IAV ( )
1966	mean	99	73***	92	150***	55***	138***	7 2	48 1
	range	80–118	40–89	53–115	113–197	44–62	92–213	4 7–10 1	18 5–100
1970	mean	97	78**	82**	121*	50***	139**	7 9	43 6***
	range	80–110	72–87	62–117	87–172	41–58	97–210	6 0–9 2	25 3–57 9

The asterisks denote the significance levels of the deviations from the normal mean values

* = 0 05 ≥ p > 0 01 ** = 0 01 ≥ p > 0 001 *** = 0 001 p

VC MVV FRC and wash out volume are given as a percentage of the predicted normal value FRC is also given as a percentage of TLC (FRC/TLC) FEV is given as a percentage of VC (FEV)

Table 6 *Reproducibility of determinations of regional lung perfusion*

The error of single determination ($\sqrt{\Sigma d^2/2n}$) is given

	n	Apical field ( )	Basal field ( )	Total lung ( )
Children without cardiopulmonary disease	12	1 3	1 4	0 8
Children with bronchial asthma 1966	13	1 6	1 0	1 2
Children with bronchial asthma 1970	13	1 8	1 9	1 4

some radiospirometric variables found in the previous investigation and in the present one imply regional abnormalities compatible with the asthma disease itself The increased standard deviations obtained by radiospirometry for the $\dot{V}$ parameters (regional ventilation) are for instance in accordance with the abnormal unevenness of overall ventilation obtained with the N_2-elimination procedure and in agreement with the abnormal regional distribution of ventilation found in adult asthmatics by i e Bentivoglio et al (1) and Hecksher et al (3) It is evident from Fig 2 that permanently decreased function of the right lung was found only in one child (for the FRC and $\dot{V}$ parameters) which supports the view that the radiospirometric abnormalities are due to the asthma disease itself[1]

To summarize the discussion so far neither clinical examination nor investigations of pulmonary function do now indicate regional organic sequelae after the recurrent pneumonia It seems therefore reasonable to be optimistic and predict that the prognosis in these children will chiefly be the same as in children with bronchial asthma which is *not* complicated with recurrent pneumonias

It can be argued that the present follow up study should include bronchographic examination Because of possible risks with bronchography it was however decided before the onset of the study that such investigation should be performed only in those children in whom the other investigations raised suspicion of local pulmonary lesion As this was not the case no child underwent bronchographic examination

The experience gained from the following of the disease in these children has given us a new approach to the question whether surgical therapy shall be carried out in them or not In some of the children the recurrent pneumonia was initially localized in the same area within the lung (mostly in the middle lobe) Furthermore chest X ray and bronchographic examination indicated atelectatic residue some months after the acute stage in some of the children Resection of the involved part of the lung was therefore discussed As the thoracic surgeons did not advise surgery our request was given up During the immediately following years these children got pneumonia in new areas of the lung Thereafter the children improved continually under conservative therapy This course of their disease and the results of the present follow up investigation show that the rejection of operation was wise Thus our approach to treatment is more conservative than that mentioned by Dees & Spock (2) in their study of middle lobe syndrome in children

We conclude that the indications of thoracic surgery in children with bronchial asthma and recurrent pneumonia shall be very restrictive

SUMMARY

14 children with bronchial asthma and recurrent pneumonia studied 1966 were reinvestigated 1970 with clinical examination and investigation of overall and regional lung function

There was a clear clinical improvement and no child had symptoms or signs of bronchiectasis or chronic bronchitis

For the children as a group investigations

[1] This child had had one attack of right sided pneumonia and one attack with involvement of both sides

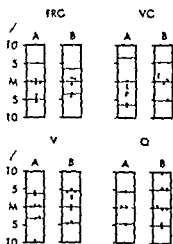


Fig 3 Regional function of the apical (*A*) and the basal (*B*) field of the right lung in 14 asthmatic children The points denote the individual values expressed as in Fig 2 The normal range ($M \pm 2$ S D of healthy children) is denoted with lines

test) The regional ventilation perfusion relation (V Q) did not differ from normal in any case

To ensure that the precision of the present investigations of regional lung function was as high as in previous investigations duplicate determinations of regional lung perfusion were performed in 13 children The error of single determination (Table 6) was of about the same magnitude as those found in previous investigations (6 8)

DISCUSSION

By story clinical examination and chest X ray the children were considered to be much healthier than 4 years ago The asthma symptoms were less pronounced and with only one exception the children had not had any new pneumonias They did not have any symptoms or signs of frequent upper respiratory tract infections chronic bronchitis or bronchectasis

However spirometry and N multiple breath procedure disclosed abnormal overall lung function and the results suggested only slight improvement, if any, compared with the examination four years ago The divergence from normal is that seen in materials of children with uncomplicated asthma investigated when the children are in a remission (4 9 10) As in the study by Kraepelien (10) the present group of children showed decreasing overinflation of the lungs and tendency to increasing FEV% The results of overall lung function are thus compatible with those expected in a group of children with asthma not complicated with recurrent pneumonia.

In the investigation 1966 (6) the results of radiospirometry suggested regional impairment of lung function due to sequelae after the recurrent pneumonias with their preferential localization to the right lung In the present study the results of radiospirometry did not show predominantly unilateral deviation from normal

The significantly large standard deviation of

Table 5 *Results of radiospirometry in the asthmatic children The values for the right lung are given For the quotients of ventilation to perfusion the values are given for both lungs*

Corresponding values in children without cardiopulmonary disease are given in parentheses

Parameter right lung	Mean ( )		S D ( )	
Perfusion (Q)				
apical	53 8	(52 4)	3 2	(2 4)
basal	54 1	(53 3)	4 6**	(2 5)
total	54 0	(53 0)	3 5	(2 7)
Ventilation (V)				
apical	49 5	(51 4)	4 0**	(1 6)
basal	55 1	(54 2)	4 7*	(2 9)
total	52 5	(53 3)	3 1	(2 0)
Ventilation (VC)				
apical	48 5	(51 6)	2 8	(3 7)
basal	53 7	(54 0)	3 0	(2 6)
total	51 6	(53 0)	2 1	(2.5)
Functional residual capacity (FRC)				
apical	50 4	(52 8)	2 7	(2 5)
basal	55 8	(55 8)	3 9	(1 8)
total	53 4	(54 9)	2 6**	(1 3)
Quotient of ventilation to perfusion (V/Q)	right lung		left lung	
	Mean ( )		Mean ( )	
apical	0 92	(0 96)	1 10	(1 00)
basal	1 03	(1 05)	0 99	(1 02)
total	0 97	(1 01)	1 04	(1 00)

The asterisks denote the significance of the deviations from the normal mean and S D values

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CASE REPORT

A CASE OF LIVEBORN TRIPLOIDY (69 XXX)

E NIEBUHR S SPARREVOHN K HENNINGSEN and MARGARETA MIKKELSEN

From the John F Kennedy Institute Glostrup the Paediatric Department of the Copenhagen County Hospital at Glostrup and the Blood Group Department Institute of Forensic Medicine University of Copenhagen Denmark

Triploidy is well known in plants and lower vertebrates but extremely rare in higher vertebrates Studying triploid rabbit embryos Bomsel Helmreich (3) found the development to be very retarded and death of the embryo before mid gestation Triploidy in man which is found in about 4 5 / of all abortions (8) appears to be compatible with life if it is present in mosaic condition (6 11 12 15 22) but not as complete triploidy Six individuals died within the first day of life (2 5 10 14 21) or in utero just before term (18) In the present paper another example of this apparently rare type of chromosome aberration is described in a liveborn infant who survived for 93 hours

CASE REPORT

The proposita a female was born October the 16th 1969 She was the second child of healthy unrelated parents the mother being 20 years old and the father 2 years The one year old brother has not shown any anomalies There was a spontaneous abortion in 1967 Otherwise nothing is known of miscarriages or children with malformations in the family No history of oral contraception exposure to radiation or infections is known Three days before delivery a low oestriol level was found in the urine and the mother was admitted to the hospital Foetal movements and heart sounds were *normal* At the 37th week of gestation, cesarean section was performed The amniotic fluid was greenish and the placenta appeared normal

The child was limp and very dysmature with large amounts of vernix caseosa The birth weight was 1 600 g, the length 41 cm She presented the following malformations (Figs 1 and 2) The head seemed large (circumference 33.5 cm) and the anterior fontanelle measured 3 × 2 cm The face was slightly asymmetrical with prominence of the left side broad nasal bridge bilateral blepharophimosis but apparently normal bulbi The ears were somewhat low set the proximal part being large with unfolded helices The left nasal cavity did not permit passage of a catheter nor did the oesophagus The chin was slightly retracted The fingers were long resembling those in arachnodactyly The two radial fingers on both hands were proximally situated and the nails were well developed Cutaneous syndactyly of the third and fourth finger on the left hand was observed. The left foot was grossly deformed with atrophic crural muscles and complete pes equino-varus In the right ankle total luxation in the talocrural as well as the subtalic joint was found There were slight contractions of the knees No clinical signs of cardiac or respiratory disease were observed

Radiological examination of the skull and extremities showed normal development and normal number of bones The hands were large The skull was normal The spine showed no malformations X ray of oesophagus showed an upper blind sac and air in the stomach indicating an oesophago-tracheal fistula

Blood sugar haemoglobin and serum calcium were normal In the urine normal excretion of 17 keto-steroids was found The child died 93 hours after birth

Necropsy findings

Increased quantities of cerebrospinal fluid and agenesis of the corpus callosum were observed Oesophageal atresia with fistula between trachea and the lower oesophageal segment was found The left lung was completely lobulated In the heart the foramen ovale was open, but otherwise no cardiac defects could be detected The gallbladder and cystic ductus were absent A normal papilla of Vater was found from which the common bile ductus could only be explored

of overall lung function still showed bronchial obstruction hyperinflation and abnormal unevenness of ventilation The degree of hyperinflation had decreased significantly since 1966 and there were fewer children with pronounced bronchial obstruction and pronounced abnormal uneven ventilation in 1970 than in 1966

Investigations of regional lung function (^{133}Xe radiospirometry) did now not indicate regional sequelae after the recurrent pneumonia

The experience gained from oversight of these children suggest that the indications of thoracic surgery in children with bronchial asthma and recurrent pneumonia should be very restrictive

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Figs 1 2 Post mortem photography of proposita Note the asymmetrical face broad nasal bridge abnormal ears and deformed hands and feet

a few centimeters Exploration gave the impression of fibrous bands continuing up into the liver which appeared normal

The pancreas and the spleen were normal The adrenals were small but normally located The kidneys ureters and urinary bladder were normal The urachus persisted The genital tract showed a normal vagina and normal uterus salpinges and ovaries

Microscopic examination of kidneys liver and lungs showed general stasis and atelectasis in the lungs

Cytogenetic studies

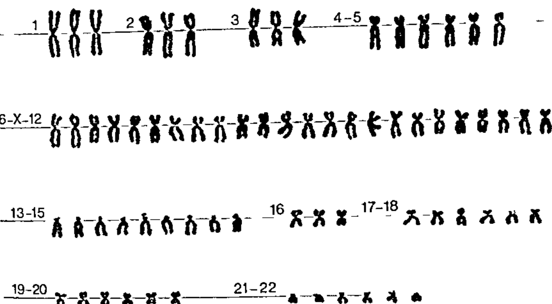
Chromosome preparations were obtained from blood lymphocytes cultured for 48 hours by a modification of the method described by Moorhead et al (17) All cells examined showed 69 chromosomes Six chromosomes were found in group G and twenty four in group C No Y chromosome was visible The findings were interpreted as triploidy with a XXX sex chromosome constitution Autoradiographic studies were carried out according to the method described by Frøland (13) In 13 cells were 2 in 5 cells was 1 and in 4 cells was no late labelling C chromosome observed The late labelling chromosomes were interpreted as X chromosomes A cell showing two late X chromosomes with and without silver grains is shown in Fig 3

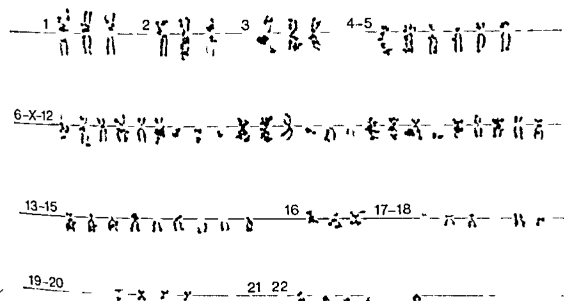
Unfortunately no sex chromatin examination or examination of skin culture was carried out before the infant died The karyotypes of both parents were normal

Serological examination

The blood groups of the patient and her parents are shown in Table 1

Fig 3 (a b) Karyotype of proposita with and without silver grains Note 69 chromosomes two late lab lling X chromosomes in group 6 X 12


1
2
3
4-5
6-X-12
13-15
16
17-18
19-20
21-22
a


1
2
3
4-5
6-X-12
13-15
16
17-18
19-20
21 22
b





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Table 4 *Liveborn infants with triploidy*

Reference		Bernard et al 1967	Edwards et al 1967	Papiernik & Berkhauer 1968	Butler et al 1969	Keutel et al 1970	Schindler & Mikamo 1970	Present case
Sex		M	M	M	F	M	M	F
Duration of pregnancy (weeks)		30	32	41	35	36	39	37
Birth weight (gram)		1800	1700	1400	1825	1850	1450	1600
Crown heel length (cm)		41	41	43	41 5	41	47	41
Birth order		2	4	?	1	2	2	2
Maternal age		28	30	?	21	22	26	20
Paternal age		39	37	?	24	24	?	22
Age at death (hours)		15	?	In utero	23	15 min	6	93
Karyotype	blood	69 XXY	?	?	69 XXX	?	69 XYY	69 XXX
	skin	?	69 XXY	69 XXY	69 XXX	69 XXY	69 XXY	?
Head circumference (cm)		32 5	32 0	?	30 5	?		33 5
Broad nose bridge		+	+	?	+	+	−	+
Epicanthus		−	−	?	−	?	−	−
Microphthalmia		?	+	?	+	−	−	−
Colobomata		?	+	?	+	−		−
Low set ears		+	+	+	−	−	+	(+)
Malformed ears		+	+	+	+	(+)	?	+
Micrognathia		+	−	?	−	−	−	(+)
Syndactyly		+	+	+	−	+	+	(+)
Simian crease		+	+	?	−	+		
Proximal situated thumbs		?	?	?		?	?	+
Foot deformity		?	−	−		+	−	+
Hypospadias			+		0	−	−	0
Bifid scrotum			+		0	−	−	0
Chryptorchidism		−	+		0	+	+	0
Heart anomaly		−	−	+	+	−	−	−
Kidney anomaly		(+)	−	−	+	( )	−	−
Hypoplasia of adrenals		?	+	?	+	+	+	−
Atresia of bile-ducts		?	−	−	−	−	−	+
Atresia of oesophagus		?	−	−	−	−	−	+
C N S malformations		?	−	?	−	+	?	+

in some of the liveborn triploids (10 21) From seven liveborn triploids with a 2N/3N mosaic (6 11 12 15 19 20 22) no clear cut clinical picture emerg d as they shared only few ab normalities such as mental retardation low birth weight syndactyly and eye defects Two of these infants died within the first 2 days of life (19 20) and the survivors were between 10 months and 10 years old at the time of publication Considering only the seven proposita with a complete triploidy (Table 4) some common features occurred suggesting a tri ploid syndrome premature babies surviving only for few hours or days with very low birth weight (mean 1 660 g) and retarded growth in length Their heads seem large but the head circumferences correspond to the gestational age The nasal bridge is broad the ears low set and slightly malformed Eye defects are occasionally found Syndactyly simian creases and in the males a small hypospadic penis are nearly inevitable features Internal ab normalities seem rather frequent but the pic ture is not uniform different organs being in volved Of particular note is the adrenal hypo plasia which was present in 5 patients in cluding our case (5 10 14 21)

Edwards et al (10) have discussed various mechanisms leading to triploidy The serolo gical examinations in our family favour the interpretation that an extra haploid set was derived from the patient s mother In 3 other cases blood group examinations or marker chromosomes suggested meiotic failure in maternal oogenesis (10 11 12) Conflicting re ports on sex chromatin in triploid cells have been given In most cases with three sets of autosomes and XXY sex complement no Barr

Table 1 *Blood groups of patient and her parents*

Patient	A_1MNS + s + (C +)[a] C^w + D + E − c + e + P_1 − K + Kpa − Fya + PGM 2-2 ac Ph B AK 1-1
Mother	OMS + s? C + C^w − D + E − c + P_1 + K − Kpa − Fya + PGM 2-2 ac Ph B AK 1-1
Father	A NS − s + (C −)[a] C^w + D + E − c + P_1 + K + Kpa − Fya + PGM 2-2 ac Ph AB AK 1-1

[a] Presence or absence of the C factor is inferred from titration experiments

No serum was available from the patient for serum typing and the small sample of cells did not allow regular dose–effect studies However, the Rh constellation offered the possibility of showing the presence of 3 allelic Rh factors in the blood of the child The red cells were strongly agglutinated by a pure anti C^w and a pure anti-c but unfortunately no pure anti C was available However, as seen from Table 2 the results of titration experiments against a serum reacting strongly with factor C and definitely weaker with factor C^w suggested the presence of the C as well as the C^w and c factors on the red cells of the patient

Table 2 *Titration experiment against an anti CC^w giving definitely weaker reactions with factor C^w than with factor C*

Cells	Test serum			
	Anti C + C^w Holyoake			
	1/1	1/2	1/4	1/8
Patient (CDe/C^wDe/cde)	+ + +	+ +	+	−
Control C^wDe/cde	+	−	−	−
— C^wDe/CDe	+ + +	+ +	+	−
— CDe/cde	+ + (+)	+	−	−
— CDe/CDe	+ + +	+ + (+)	+	−

Table 3 *Agglutination reactions against a human anti M and a diluted rabbit anti N*

Cells	Test serum			
	Anti M 7751		Anti N 105	
	1/3	1/6	1/12	1/80
Patient (M/M/N)	+ +	+ (+)	−	(+)
Control M/M	+ +	+ (+)	−	/
Control M/N	(+)	−	−	+
Control N/N	/	/	/	+ + +

From Table 3 it appears that the red cells of the patient give a double dose reaction against a human anti M and a single dose reaction against a diluted rabbit anti N Thus the results suggest that the patient's MN and Rh genotypes are M/M/N and CDe/C^wD cde and that at least in these two systems 3 allelic genes have been able to express themselves in the phenotypes Further the MN reactions indicate that the duplication has taken place in the maternal gamete

DISCUSSION

It might be discussed whether in the present case a 2N/3N mosaic exists as only lymphocyte cultures were carried out However as shown by Atkins et al (1) triploid cells in man are at a disadvantage as compared with diploid cells and this taken together with the fact that in all cases where a mosaic has been found lymphocyte cultures showed either a diploid cell line (7 11 12 22) or a 2N/3N cell line (15) makes it reasonable to assume a complete triploidy in our case as no mosaic could be detected in the blood cultures The agglutination of all cells with the previously mentioned Rh sera also support this interpretation

In contrast to the retarded but otherwise harmonious and normal development in triploid rabbit embryos (3) most specimens of human triploid abortions were severely macerated or did not contain a recognizable embryo (9) The most striking feature of triploid abortions were hydatidiform degeneration in the placenta This has been found in about half the reported cases and was also present

Table 4 *Liveborn infants with triploidy*

Reference		Bernard et al 1967	Edwards et al 1967	Papiernik & Berkhauer 1968	Butler et al 1969	Keutel et al 1970	Schindler & Mikamo 1970	Present case
Sex		M	M	M	F	M	M	F
Duration of pregnancy (weeks)		30	32	41	35	36	39	37
Birth weight (gram)		1800	1700	1400	1825	1850	1450	1600
Crown heel length (cm)		41	41	43	41 5	41	47	41
Birth order		2	4	?	1	2	2	2
Maternal age		28	30	?	21	22	26	20
Paternal age		39	37	?	24	24	?	22
Age at death (hours)		15	?	In utero	23	15 min	6	93
Karyotype	blood	69 XXY	?	?	69 XXX	?	69 XXY	69 XXX
	skin	?	69 XXY	69 XXY	69 XXX	69 XXY	69 XXY	?
Head circumference (cm)		32 5	32 0	?	30 5	?	?	33 5
Broad nose bridge		+	+	?	+	+	+	+
Epicanthus		–	–	?	–	?	–	–
Microphthalmia		?	+	?	+	–	+	–
Colobomata		?	+	?	+	–	+	–
Low set ears		+	+	+	+	–		(+)
Malformed ears		+	+	+	+	(+)	?	+
Micrognathia		+	–	?	–	–		(+)
Syndactyly		+	+	+	–	+	+	(+)
Simian crease		+		?	+	–	+	
Proximal situated thumbs		?	?	?	+	?	?	+
Foot deformity		?	–	+	–	+	–	+
Hypospadias		+	+	+	0	–	+	0
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Chryptorchidism		+	+	–	0	+	+	0
Heart anomaly		–	–	–	+	–	–	–
Kidney anomaly		(+)	–	–	–	(+)	+	–
Hypoplasia of adrenals		?	+		+	+	+	+
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Atresia of oesophagus		?	–	–	–	–	–	+
C N S malformations		?	–	?	–	+	?	+

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— CDe/cde	++(+)	+		−
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Control M/N	(+)	−	−	+
Control N/N	/	/	/	+++

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CASE REPORT

INFANTILE LOBAR EMPHYSEMA WITH LOBAR AGENESIA AND CONGENITAL HEART DISEASE

POUL STRUNGE

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The syndrome of infantile lobar emphysema (ILE) occurs in neonates and infants The predominant clinical feature is progressing respiratory distress which without treatment may lead to cardiorespiratory failure The respiratory symptoms are due to the development of emphysema in a localized area of the lung resulting in compression atelectasis of adjacent lobes as well as displacement of the mediastinum Often it is not possible to demonstrate the cause of the localized emphysema In some cases there is localized obstruction of a main bronchus or one of its branches due to occlusion of the lumen changes of the bronchial wall or pressure by extrabronchial structures (4 10 11 14)

Within the past two years we have treated two infants with ILE associated with types of congenital heart disease not previously reported

CASE REPORTS

Case 1 Newborn boy his parents first born at term after a normal pregnancy Delivery uneventful birth weight 3 100 g No abnormalities at birth Apgar score 10 at one minute At the age of two hours his respiration was a little wheezy and his voice hoarse Auscultation of the heart revealed a faint systolic murmur An ECG showed a few ventricular premature beats During the following few days he developed increasing dyspnoea and cyanosis on exertion and when placed on the right side Chest films showed the right lung field to be more radiolucent than the left. On the seventh day of life the condition deteriorated The ECG showed sinus tachycardia (200/min) Digitalis and ampicillin were administered Following temporary improvement there was further progression and eventually considerable doming of the right hemithorax A chest film now showed hyperaeration of the right lung with herniation into the anterior mediastinum *displacement of the heart to* the left and infiltration of the right lower and upper lobes as well as of the entire left lung Following intubation and aspiration from the right main bronchus, the right sided emphysema yielded and the mediastinal shift was abolished The clinical condition improved temporarily but on the 15th day of life the patient had to be re intubated and placed on a respirator because of decreasing alveolar ventilation The patient deteriorated and convulsions, recurrent pulmonary infiltration and increasing cardiac dilatation supervened *He died at the age of 19 days from* cardiac arrest

Autopsy disclosed the presence of truncus arteriosus and a ventricular septal defect The two pulmonary arteries (PA) originated separately from the arterial trunk. The left PA was found to be normal whereas the right one was distinctly enlarged passing posterior to the ascending aorta and descending anterior to the right main bronchus The ductus arteriosus was closed The left lung consisted of one lobe only the right one of an upper and a lower lobe The lung tissue was pale nodular with scattered atelectases There was no abnormality of the trachea or bronchial tree A small atrophic thymus was found

Microscopic examination revealed atelectatic areas, wide interalveolar septa and congestion of the pulmonary vessels In a few areas there were air filled alveoli and subpleurally emphysematous thin walled and partially destroyed alveoli were recognized No inflammation or hyaline membranes Presumably the microscopic appearance of the lungs was influenced by the respirator therapy In the thymus there was *hypoplasia of the lymphoid tissue and a highly in*creased number of Hassall s corpuscles but no fibrosis A similar hypoplasia of the lymphoid tissue was observed in the spleen and in a *lymph node*

bodies were detected, findings which appear to be at variance with those of Keutel et al (14), Rumpler et al (19) and Sacrez et al (20) Sex chromatin positive cells with 2 Barr bodies were demonstrated by several authors (4 5, 10, 16) in triploids with a XXX complement Autoradiographic studies in a 69,XXY cell line (1) showed absence of a heterochromatic X chromosome

The presence of two late labelling X chromosomes (59%) one hot X (23%) or unlabelled X chromosome (18%) in this report is in agreement with the findings in 47 XXX females

SUMMARY

A case of complete triploidy is described in a female born after 37 weeks gestation She died at 93 hours of age and presented the following abnormalities Low birth weight, severe physical retardation broad nasal bridge malformed ears micrognathia syndactyly and foot deformities

Various other defects were found at autopsy Autoradiographic studies showed a mixture of one two or no heterochromatic X chromosomes Serological data suggested meiotic failure in maternal oogenesis

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Key words Chromosome aberrations complete triploidy congenital malformations

angiocardiography seem to afford a more direct approach to an etiological diagnosis (9 12)

In case 1 and 2 it was found a truncus arteriosus and a valvular stenosis of the aorta respectively These cardiac malformations have not previously been reported in association with ILE. On the other hand ILE has been described in combination with ventricular septal defect persistent ductus arteriosus coarctation of the aorta and the tetralogy of Fallot (2 5 7 11) In a number of cases the pathogenesis of the emphysema is presumed to involve bronchial compression due to an increased pressure with dilatation of the homolateral pulmonary artery (11) In case 1 a distinctly enlarged right PA was anatomically related to the right main bronchus Maybe more interesting in case 1 the right lung was unique in consisting of only two lobes and this anomaly may better explain the development of the lobar emphysema than did the enlarged PA In case 2 there was no definite anatomical or haemodynamic explanation of the emphysema

Whenever ILE is due to congenital heart disease long term treatment must of course depend upon the nature of the heart disease and the possibilities of surgical correction Most authors have accepted lobectomy as the standard treatment (1 3 5 7 8 9 10) Jones et al (5) found that the immediate problem was more often respiratory than cardiac and felt that lobectomy must be considered as the primary treatment Leape & Longino (8) even suggested that lobectomy should be carried out within 24 hours of the diagnosis with a view to obviating the risk of mediastinal displacement

On this backgound it is of great interest to investigate possibilities of primary relief by more conservative measures In our case 1 prompt but temporary relief of the emphysema was obtained by catheterization of the right main bronchus Especially with right sided emphysemas in which bronchial catheterization is easier this procedure should be considered as a palliative step facilitating further studies such as cardiac catheterization and angiocardiography

(A third case in our department a newborn boy without heart disease who developed a pronounced right sided middle lobe emphysema a few hours after birth was treated with intubation and with permanent relief)

At autopsy the right lung of patient 1 was found to consist only of an upper and a lower lobe and the left one of only one lobe No other gross abnormalities were found Such lobar agenesia is uncommon (6) and bilateral lobar agenesia does not appear to have been reported previously Supernumerary lobes have been described in some patients with congenital heart disease but do not appear to have been associated with truncus arteriosus (12) The thymic atrophy and lymphatic hypoplasia found in this patient may be interpreted as accidental involution owing to the patient's poor condition (6 13)

SUMMARY AND CONCLUSIONS

Two infants with lobar emphysema (ILE) are described The emphysema was apparently secondary to congenital cardiac malformations truncus arteriosus and valvular aortic stenosis neither of which has been previously reported in association with ILE In one patient postmortem examination of the lungs disclosed bilateral lobar agenesia which anomaly maybe plays an etiological role for the development of the emphysema in this case

In patients with congenital heart disease and complicating ILE the presenting symptoms may be pulmonary rather than cardiac In most cases chest radiography will disclose the emphysema but given the suspicion of heart disease early cardiac catheterization and angiocardiography should be considered

In the acute stage catheterization of the main bronchus concerned may bring immediate relief If this treatment proves unsuccessful lobectomy may be necessary



Fig 1 Angiographic findings in a case of infantile lobar emphysema (The contrast was injected through a catheter placed in the right atrium filling the right ventricle and the pulmonary artery) The vessels in the right middle lobe are sparse stretched and spread apart whereas in the upper lobe they are displaced and compressed In the left lung there is a rather good filling of the vessels

Case 2 Newborn girl her parents first born at term after a normal pregnancy Delivery uneventful birth weight 3 450 g Apgar score 10 at one minute Immediately after birth a systolic murmur was heard However the baby showed a satisfactory post natal weight gain and the ECG and chest radiogram were normal At the age of five weeks she was re admitted because of severe pallor and tachypnoea Physical examination revealed voussure systolic murmur subcostal retractions enlarged liver and impalpable femoral pulsation Chest films showed an ectatic but normally positioned heart The right middle pulmonary lobe was radiolucent and hyper aerated whereas the remaining areas of the right lung appeared dense The patient was given digitalis and ampicillin In the course of a few days the condition improved and the radiological signs of emphysema disappeared As the patient now was without respiratory symptoms a cardiac catheterization was performed revealing a normal pressure in the pulmonary artery and in the right ventricle and atrium No shunts were demonstrated Unexpectedly the pulmonary angiocardiography showed pronounced emphysema of the right middle lobe which protruded into the anterior mediastinum and displaced the mediastinal structures towards the left (Fig 1) There was a valvular aortic stenosis with poststenotic dilatation and left ventricular hypertrophy The ductus arteriosus was closed and the aortic arch appeared normal On the day after the cardiac catheterization the patient died from acute respiratory failure

Autopsy confirmed the presence of aortic stenosis with thickened cartilaginous bicuspid valves. Slight enlargement of both atria The pulmonary artery appeared normal as did the trachea and main bronchi

DISCUSSION

Clinically, the ILE is characterized by varying degrees of cardio pulmonary failure showing tachypnoea cyanosis chest asymmetry hyperresonance on percussion and weakened respiratory sounds over the affected hemithorax (4 8 9 10) The diagnosis is generally based upon the findings on plain chest films, the emphysema being usually visualized as a translucent area of the lung field often extending into the mediastinum and associated with displacement of the mediastinal structures towards the contralateral side (4 8 10) In our two patients the emphysema developed in the right middle lobe but the left upper lobe is affected just as often (5 9 10 11). Stanger et al (11) suggested that this could be explained by the close anatomical relationship of the right and left pulmonary arteries (PA) to the right middle lobe bronchus and left upper lobe bronchus respectively Reviewing the literature, these authors too, found 12 cases of ILE in the RML and eight in the LUL in patients with congenital heart disease and an elevated pressue in PA among patients with a normal pressure in the PA they found two cases only in the LUL none in the RML

Our experience from case 2 indicates that a plain chest film does not always afford sufficient information Pulmonary angiocardiography revealed extensive middle lobe emphysema on the right side with marked mediastinal shift (Fig 1) which was difficult to discern on the plain film The possibility of a relapse not clinically apparent seems unlikely

Bronchography too has been considered but is rejected by many authors as being unnecessary or dangerous (3, 5 8 10 15) The cause of the obstruction is not always demonstrable In selected cases cardiac catheterization and

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CASE REPORT

CHRONIC LYMPHOCYTIC LEUKEMIA IN AN INFANT

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Chronic lymphocytic leukemia is a disease rarely reported in children Some authors do not even recognize the existence of this dis order in childhood (8 9) A case of chronic lymphocytic leukemia in an infant gives special diagnostic and therapeutic problems The following report may illustrate these problems

CASE REPORT

A 9 month-old boy was admitted to hospital in September 1968 for investigation of splenomegaly He was the first child of young healthy parents with no family history of blood di orders or malignant dis ase Prenatal and perinatal periods were unevent ful birthweight 3 700 g len th 50 cm Weight gain and development w re normal No signs of dis ase were present and th re was no exposure to X ray antenatally or in the infant period 3 weeks before adm ss on there was loss of app tite and a slight dy pepsia Th mo her consulted h r general practi tioner An abdominal mass was found and th pati nt sent to hospital On admission th patient gave no signs of disease except for a smooth firm tumour in the left side of the abdomen with an extension from the curvature to the iliac crest (*Fig 1*) Th re was no peripheral lymph node enlargem nt and no signs of anemia or hemorrhagic diathesi

Blood tests showed a S R 63 mm hr slight anemia (Hb 10 g 100 ml) with M C V 68 μ M C H C 31 g 100 ml reticulocytes 4 4 and leucocytosis (3 300 to 64 300 microlitre) with 80–85% lympho cytes in th blood smear (Fig ?) There was no thrombocytopenia or signs of h molysis (serum bi lirubin serum haptoglobin serum iron and erythrocyte fragility test were normal) direct Coombs test negative) Two marrow specimens with an interval of 4 weeks showed l ke the peripheral blood an increase in number of lymphoid cells mature dif ferentiated relatively small lymphocytes with a few larger more immature in between Few stem cells were present there was normal erythropoiesis and granulocytopoiesis For distribution or marrow cells see Table 1

The diagnosis as acute infectious lymphocytosis was suspected there being no basis for diagnosing anoth r known infection and the patient was observed without treatment During the following pe riod leucocytosis was unchanged (Fig 2) slight ane mia p rsisted and the patient was in good h alth A third marrow examination was unchanged chromosome analysis of peripheral blood and X ray of long bones were found to be normal Paul Bunnell test cytom galovirus titre Wassermann and toxoplasmosis reactions were negative and S G O transaminase fractionated serum proteins and serum immunoglob ulins were normal for the age (serum total protein 7 2 g/100 ml serum gammaglobulin 0 92 g/100 ml)

After several months observation the state was unchanged and the chronic lymphocytic leukemia of adult type likewise A trial treatment with a small dosis of prednison (5 mg p r day) and 6 mercap topurin (25 mg per day) was started This was fol lowed by splenectomy in March 1969 leu ocytosis and splenomegaly being unchanged after 2 months medical therapy Enlarged mesenterial lymph nodes were revealed at th operation Th liver seemed normal Weight of the spleen was 195 g Microscopy of the spleen showed a picture dominated by lympho blasts and lymphocytes building up zones round the trabeculae in places confluence of these zones Lymphoblasts and lymphocytes with intact reaction cent rs were found in th lymph nodes Th his tological pi ture in accordance with lymphogenic leu k mia 8 weeks after the operation cystostatic and pre inison treatment were discontinued

During the following out patient control the pa tient was found to be in normal development There was a tendency to infection in th upper airways

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patient showed no signs of disease Blood ex amination showed a constant leucocytosis (16 900 to 115 000 leucocytes/microlitre) with lymphocytosis The marrow was dominated by mature differentiated lymphocytes The fol lowing clinical course was characterized by a good general condition slight anemia and unchanged leucocytosis There was no improve ment of blood status after treatment with prednison and 6-mercaptopurin or the follow ing splenectomy 10 months after the first ad mission cervical and axillary adenitis and a light hepatomegaly developed Treatment with cyclophosphamid 50 mg every second day was started. After 12 months a fulminant sepsis combined with shock occurred followed by death of the patient

DISCUSSION

Most childhood leukemias are characterized by abnormal growth of undifferentiated cells *This* is the case in stem cell leukemia as in the chronic myelogenous leukemia The chronic forms make up less than 5° of all cases of leukemia in children (11) and it is *then the* chronic myelogenous leukemia (1 3 and 7) In the literature *isolated case reports* of chronic lymphocytic leukemia in childhood are found (2 6) Holowach *examined a series* of published cases and pointed to the fact that the diagnosis in some cases was questionable being made without accurate description of the cell morphology *In Videbæk s thesis 68* patients with chronic lymphocytic leukemia are mentioned among *these 2 children of* 4 and 11 years (10) Lymphosarcoma with leu kemic tranformation is *sometimes d scribed* as lymphatic leukemia but in childhood how ever this disease is characterized by a pri marily normal leucocyte count and a later de velopment of a blood picture *similar to* that of stem cell leukemia (7 8)

Our patient presented few *symptoms* and none characteristic of stem cell leukemia Dif ferent examiners independently agreed that the lymphocytes of the blood smears looked mature and were of normal size with dense aggregates of nuclear chromatin and only a few larger cells containing a nucleolus or fragile nuclei Nor were marrow aspirates dom inated by stem cells which were only found singly whereas the lymphocyte per cents were between 48 and 79

During the first months the clinical picture was consistent with chronic infection with ab- solute and relative lymphocytosis Examina tions to confirm this were negative The pro- longed leucocytosis and the splenomegaly were not compatible with acute infectious lymphocytosis according to Wintrobe (11)

The criteria for making the diagnosis leu kemia in our patient were not clear until af ter the prolonged clinical course The symp- toms and the cell morphology were those of a chronic lymphocytic leukemia in adults No chromosomal aberration was demonstrated which corresponds to the findings in chronic lymphocytic leukemia (11) Stem cells could not be found in the peripheral blood at any time

Choice of treatment was not obvious After some months uneventfull clinical course we made a trial treatment of prednison and 6- mercaptopurin in small doses though not of chlorambucil to avoid producing irreversible marrow depression The indication for splen ectomy was the excessive splenomegaly The *risk of post* splenectomy infections was related to the risk of infection due to the impair ment of antibody response seen in chronic lymphocytic leukemia and splenectomy was performed because of the age over 1 year (5) In adults recurring infections due to immune deficiency ultimately terminate life in chron ic lymphocytic leukemia (11) The immuno- suppressive treatment and the postsplenectomy state may have contributed to the fulminant final course in our patient

The malignance of the condition was per haps over estimated Darte et al (4) recently published a case report of congenital lym phoid hyperplasia with persistent hyper lymphocytosis in a normally developing pa

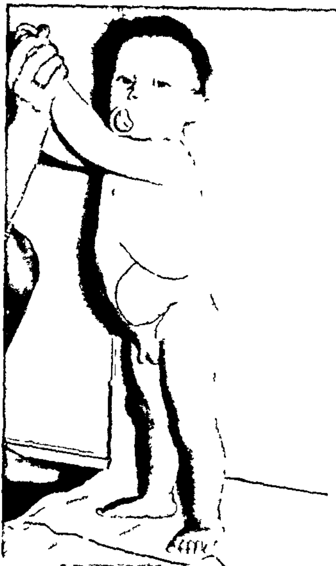


Fig 1 The patient 14 months old Splenomegaly is shown

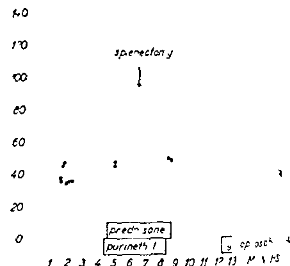


Fig 2 Leucocyte count during clinical course

as during the preoperative period A peripheral lymph node enlargement and moderate hepatomegaly were observed in the summer of 1969 Leucocyte count was increasing and in mid September treatment with cyclophosmamid 50 mg every second day was started On the 23rd of September the patient was admitted acutely ill with shivering fever and unstable respiration The clinical picture was one of sepsis with irreversible shock and in spite of intensive treatment with antibiotics steroids digitalis and transfusion the patient died 8 hours following re admission A blood culture shortly after re admission showed no growth of bacteria On examination of material from the necropsy a few coli and a few proteus Morgagni were found in the cardiac blood The autopsy showed single enlarged lymph nodes in the mediastinum along the inferior caval vein and at the porta hepatis At microscopy the lymph node structure had disappeared and was replaced by lymphocyte like cells without formation of germinal centres In the liver were found infiltrates of small dark uniform cells The bone marrow was hyperplastic with scattered foci of lymphoid tissue without formation of germinal centres Lungs heart thymus kidneys adrenals and intestinal canal were normal and intact

Summary

9 month old boy admitted for splenomegaly following a few weeks with mild dyspeptic symptoms Apart from an enlarged spleen the

Table 1 *Distribution of bone marrow cells in per cent*

	Stem cells	Promyelo cytes	Myelo cytes	Meta myelo cytes	Granulo cytes	Lympho cytes	Mono cytes	Erythro blasts	Normo-blasts
Marrow 1 (Oct 1968)	2		7	4	8	68	1	2	8
Marrow 2 (Nov 1968)		2	22	5	4	48		10	9
Marrow 3 (Jan 1969)	4		2		5	79		3	7

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CASE REPORT

INTRAMURAL CALCIFIED FIBROMA OF THE HEART

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Primary tumours of the heart are very rare The reported incidence in autopsy materials varies widely Straus & Merliss (10) found only 8 cases in 480 331 autopsies performed in the United States from 1938 to 1942 (0 0017%) Other authors have found a higher incidence the highest reported being that of Shelburne (8) who found 3 cases among 1 200 autopsies (0 25%) Myxomas comprise about 50% rhabdomyomas 20 % and sarcomas 20% of the total The remaining 10 % consist of fibromas lipomas and angiomas Because of confusion in nomenclature it is difficult to enumerate all published cases of intramural fibroma With this reservation we have been able to collect 53 cases from the literature up to the end of 1970 43 of these tumours occurred in infancy and childhood The patients showed great variations in the clinical picture and therefore the tumour diagnosis has most often been made at autopsy It is desirable to report the incidence of these rare tumours in order to assist early clinical recognition and possible surgical treatment of future cases

The observation of myocardial calcifications has been described on pathological examination in different diseases of infancy and childhood including heart tumours The calcifications are however usually diagnosed by post mortem examination and are very rarely observed *in vivo* Thus in only 7 of the reported 53 cases of intramural fibroma have calcifications been visible on chest roentgenograms (1 4 5 7 11) The present paper is the case report of an infant with an intramural fibroma of the heart in which calcifications of an unusual pattern were clearly visible on the roentgenological pictures of the chest

CASE HISTORY

The patient was a 7 month-old girl When she was 5 weeks old she had a febrile illness with weakness and anorexia of 2 weeks duration At the age of 2 months sh was admitted with mild signs of otitis Roentgenological examination of the chest at that time showed a slightly enlarged heart but was otherwise normal In the following months she was weak and hypotonic but her mental development was normal

At 5 months she had a second febrile illness with moderate respiratory difficulty and was treated with penicillin After a few days she was admitted for th second time The clinical examination showed moderate liver enlarg ment and a soft uncharacteristic cardiac murmur The blood pressure was normal both in the upper and lower extremities Ophthalmoscopic examination was normal

Laboratory investigations showed Hb 13 6 g/100 ml hematocrit 43 vol% and WBC 9 000 with a normal differential count ESR was 14 15 and 22 mm Urinanalysis was normal Serum proteins with electrophoresis and immunoelectrophoresis erum Ca and P were normal Repeated blood cultures and examinations of blood and stools for virus were negative Coxsackie B 1–6 virus antibodies and mumps antigen CBR were negative Dye test was normal but hemagglutination with toxoplasma antig n and cytomegalovirus antibodies were possibly elevated

Roentgenological examination of the chest at this time showed a generalized enlarg ment of the heart with thin stripes of calcification within the cardiac

tient who was observed for 7 years without any specific treatment

SUMMARY

Chronic lymphocytic leukemia is very rarely seen in childhood This is a case report of a boy 9 months old in whom the diagnosis was made according to the clinical course and cell morphology The diagnostic and therapeutic considerations are discussed

ACKNOWLEDGEMENT

I am grateful to Dr E A Larsen C Johansen and E Bredahl Bispebjerg Hospital and to Professor Dr Aa Videbæk Gentofte Hospital for describing and discussing the marrow preparations

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Fig 4 Roentg nological picture of the isolated heart with calcifications

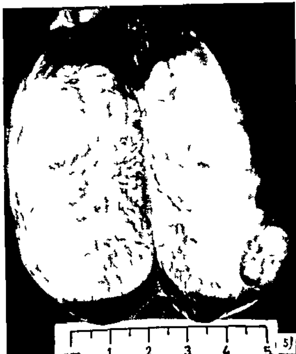


Fig 5 Macroscopic picture of the heart with the cut surface of a large circumscribed non-encapsulated tumour in the interventricular septum

cellular with spindle shaped fibroblasts criss crossing in an irregular fashion and in other places it was less cellular with dense collagenous tissue The calcifications were most pronounced in the latter areas The muscle cells within the tumour had a normal appearance and were mo t numerous in the periphery of the tumour but were also found in more central areas (Fig 6) The tumour had no capsule but was surrounded by an area of compressed heart muscle No spider cells could be detected Stains for glycogen mucin and elastic fibres were negative Fig 7 shows a cellular area with fibroblasts and in the lower l ft corner striated muscle cells are seen A small black area of calcification is also shown There was no evidence of malignancy The lungs showed signs of chroni and acute passive congestion The skin was normal and examination of the nervous system revealed no signs of tuberous sclerosis

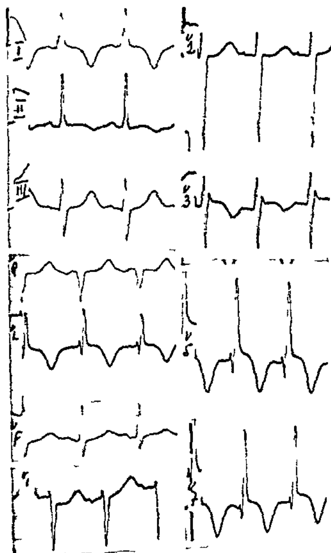
DISCUSSION

The macroscopic and microscopic appearance of the tumour was consistent with that of an intramural fibroma of the heart The bulk of knowledge about this tumour is derived from published case reports In the literature the term fibroma has been used most frequently (1 6) but some authors use other terms such as elastofibroma elastomyofibroma rhabdomyoma rhabdomyofibroma fibrosarcoma mesoblastoma mesenchymoma nodular fibroelastosis and hamartoma (see (1) for references) This confusion of terms reflects an underlying controversy over the pathogenesis The tumours have been regarded as true fibromas tumourlike malformations (hamartomas) or true neoplasms originating from the primitive mesenchyme of the heart Pathologically they seem to constitute a fairly homogeneous group consisting of interlaced collagenous and elastic fibres admixed with fibroblasts and occasional cardiac muscle cells Until the question of their pathogenesis has been settled it is suggested that the descriptive term of fibroma is used inasmuch as pathologically they bear a striking resemblance to fibromas both macroscopically and microscopically

In most of the published cases the fibromas



Figs 1 2 Anteroposterior and lateral views of the chest showing a generalized enlargement of the heart with thin stripes and whorls of calcifications within the margins of the cardiac silhouette They were not visible on chest roentgenograms taken 3 months earlier



silhouette (Figs 1 and 2) Roentgenograms of the skull were normal

Electrocardiography showed signs of marked left ventricular hypertrophy with inverted T waves and slightly elevated ST segments in the left precordial leads (Fig 3) Cardiac catheterization had to be interrupted because the child had an attack of ventricular fibrillation when the catheter was introduced into the right atrium Therefore no information was obtained on intracardiac pressures or oxygen tension and angiocardiography could not be performed The fibrillation was rapidly cured by defibrillation

The patient s hypotonia and weakness increased Roentgenograms of the chest showed increasing calcification within the cardiac silhouette She developed cardiac insufficiency which was treated with digitalis When 7 months old she had attacks of ventricular fibrillation which did not respond to defibrillation and she died

Post mortem examination showed an enlarged heart weighing 130 g with a large circumscribed non encapsulated tumour in the interventricular septum bulging into both ventricles (Fig 5) It measured 6 × 4 × 4 cm and on sectioning it had an appearance very reminiscent of a uterine fibromyoma An X ray picture of the isolated heart showing the calcification within the tumour is seen in Fig 4 Microscopically the tumour consisted of fibrous tissue in which small groups of striated muscle cells and areas of calcification could be found In som places the tumour was

Fig 3 Electrocardiogram showing signs of left ventricular hypertrophy with inverted T waves and slightly elevated ST segments in the left precordial leads



Fig 4 Roentg nological picture of the isolated heart with calcifications

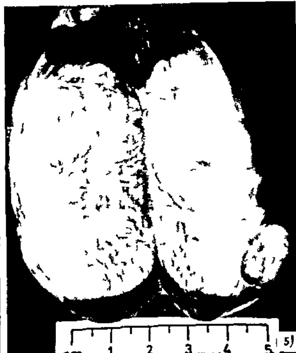


Fig 5 Macroscopic picture of the heart with the cut surface of a large circumscribed non-encapsulated tumour in the interventricular septum

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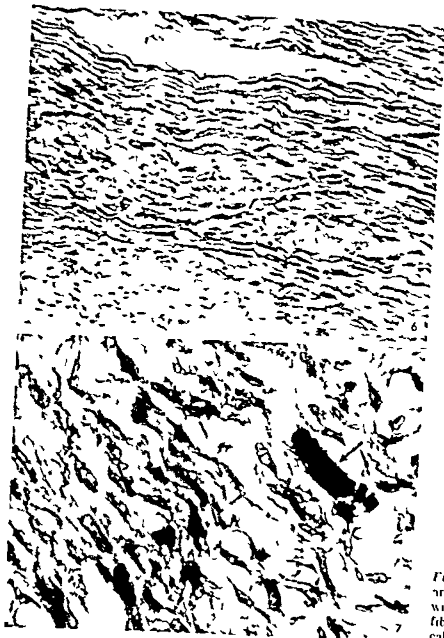


Fig 6 Microscopic picture of the margin of the tumour showing strands of heart muscle cells within the periphery of the tumour PTAH ×175

Fig 7 Microscopic picture of a central area of the tumour showing muscle cells with cross striations collagen fibres and fibroblasts and a small area (⟶) of calcification H+E ×490

have occurred in children under the age of 6 years and in many cases in neonates and in infants only a few months old Fibromas of the heart are located most often in the anterior and lateral wall of the left ventricle more seldom in the interventricular septum as in the present case Only very rarely have they been found in the right ventricle or in the posterior wall of the left ventricle (1)

The location and size of the tumour will affect the clinical picture The tumour may be completely symptomless and may be an incidental finding at autopsy in patients who have died from other causes or may itself be the cause of sudden death (1) Review of the literature shows that in most cases the correct diagnosis was made at autopsy and was not even suspected during the patient s lifetime Indeed heart tumours are the great imitators of cardiovascular disease counterfeiting almost any hemodynamic syndrome and even producing symptoms and signs from other organs thus directing the attention away from the heart (2) As in the present case there may be symptoms of cardiac insufficiency and disturbances of cardiac rhythm probably from interference with the conducting system In many cases cardiomegaly occurs If the tumour is located near one of the valves a murmur may be heard Cases with symptoms of valvu

lar stenosis have been described Electrocardiography may show a variety of changes including arrhythmias bundle branch block signs of ventricular hypertrophy and occasional myocardial infarction patterns

Roentgenological examination of the chest may show diffuse enlargement of the heart or more localized abnormalities in the cardiac contour (9) As in the present case calcifications are sometimes seen appearing as thin stripes and whorls of calcium deposits within the cardiac silhouette (11) Contrast studies of the heart are probably the most precise and accurate methods available for an anatomic diagnosis of cardiac tumours (9) The tumours may produce filling defects in the contrast shadow or dislocation of the coronary arteries The diagnosis of cardiac tumour was considered in the present case but could not be confirmed because angiocardiography had to be omitted

In clinical work the most important differential diagnoses are congenital heart disease endocardial fibroelastosis glycogen storage disease (type II Pompe s disease) pericarditis myocarditis and diseases which may be accompained by myocardial infarction pattern by electrocardiographic examination

Until the advent of modern heart surgery treatment was of no avail and ante mortem diagnosis rare (3) In some of the cases of cardiac fibroma in infancy and childhood a clinical diagnosis has been made while the patient was alive Occasionally surgical removal has been *attempted* in a few cases with success The histologically benign character of the fibromas makes them accessible to treatment by the modern techniques of open heart surgery Every attempt should therefore be made to arrive at a *clinical* diagnosis in cases of obscure heart disease Unless the possibility of a *cardiac tumour* is considered diagnosis will be made at autopsy and not by the clinician In particular roentgenologically visible calcifications *of* the pattern described in the present paper should arouse suspicion of a cardiac fibroma

SUMMARY

A case of intramural fibroma of the heart in a 7 month old girl is presented Roentgenological examination of the chest showed thin stripes and whorls of calcium deposits within the margins of the cardiac silhouette and on post mortem examination these calcifications were found to be located in the cardiac tumour

The clinical picture and morbid anatomy of intramural fibromas of the heart is discussed A primary mural tumour of the heart should be suspected in children with unexplained cardiac failure or dysrhythmia intracardiac calcifications irregular heart shadows on the roentgenograms or unexplained newly developed cardiac symptoms and murmurs Angiocardiography is the best method of confirming the diagnosis

Including the present case 54 cases of heart fibromas have been described Only 10 cases were observed in adults and the fibromas mainly occur in children under 6 years of age

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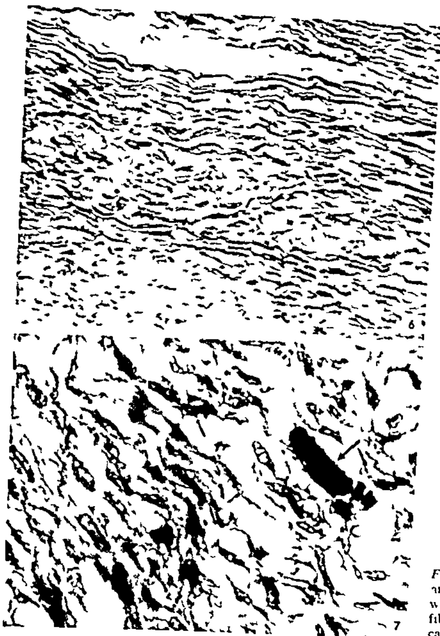


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CASE REPORT

FEMALE PSEUDOHERMAPHRODITISM ASSOCIATED WITH MULTIPLE CONGENITAL MALFORMATIONS

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Female pseudohermaphroditism connotes a condition in which the gonads are ovaries but the appearance of the external genitalia is sufficiently ambiguous to give rise to uncertainty as to the sex It is well documented that androgenic hormones can masculinize the female fetus The degree of masculinization of the external genitalia depends primarily on the stage of sexual differentiation when exposed to the androgens and may vary from slight clitoral enlargement to complete masculinization with phallic urethra (6) The androgens may have their origin in the fetus itself as in most variants of congenital virilizing adrenal hyperplasia or the hormones may stem from an androgen producing tumour in the mother The androgens may also be of exogenous orı in such as drugs given to the mother during pregnancy (7)

Rarely abnormal genital development is seen without any known exposure of the fetus to androgenic hormones Such abnormalities may occur either as an isolated phenomenon or in association with other congenital anomalies (4 5)

The present report describes a case in the latter category

CASE REPORT

An infant born to a ?0-year-old healthy woman at 36 weeks gestation was admitted to the Children s Hospital in Bergen 30 min after delivery

The family history was non-contributory The mother was a primipara and the pregnancy had been uncomplicated She gained 10 kg weight and showed no signs of virilization during pregnancy Except for iron supplement, no drugs were used The birth weight was 2 240 g and the length 44 cm The infant was cyanotic with faint respiratory movements and died 3 / hours after birth

Before death the following anomalies were noted epicanthus anteverted nostrils depressed nasal bridge broad hands with laterally displaced thumbs and limited abduction of the hip joints A nasogastric tube met resistance 10 cm down from the nostrils The external genitalia were abnormal with a very small phallus a partially bifid scrotum and no palpable gonads (Fig 1) The urethral meatus was located on the tip of the glans There was anal atresia without anal dimple A wart like prominence was seen in the coccygeal region

At autopsy the findings were bilateral pulmonary atelectasis and persistent ductus arteriosus but no cardiac abnormalities There was atresia of the oesophagus with a fistula from the trachea to the lower segment of the oesophagus On the major curvature of the gastric ventricle there was a pea sized sessile tumour which on microscopic examination revealed pancreatic tissue The colon ended in a blind pouch in the pelvic region No kidneys renal arteries ureters or bladder could be found on gross examination In the right inguinal region there was a tumour which resembled testis but microscopic examination revealed immature glomeruli and renal tubular structures (Fig 2) The adrenal glands were considered normal both on gross and microscopic examinations The phallus had a normal glans and a central urethral meatus On microscopy the presence of a phallic urethra was confirmed and likewise cavernous bodies were demonstrated (Fig 3) A normal looking uterus and Fallopian tubes were present The ovaries were found in the normal position and were histologically normal for age (Fig 4) Strands of fibrous tissue extended from the cervical region of the uterus towards the perineal region The wart like body in the coccygeal region was connected with the medullary canal by fibrous

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Key words Fibroma heart tumour chest calcifications

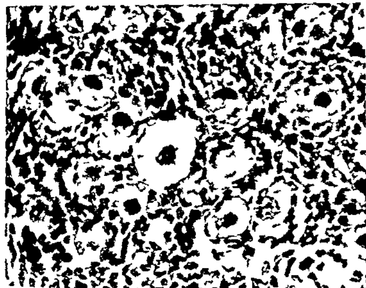


Fig 4 Photomicrograph of the ovarial cortex showing numerous primordial follicles H E × 370

genous teratogenic factors may interfere with differentiation of the external genitalia

SUMMARY

The clinical and pathological findings in a newborn female pseudohermaphrodite with multiple congenital malformations are reported The infant had a phallus with a centrally located urethra and bifid empty scrotum Ovaries Fallopian tubes and uterus were demonstrated at autopsy She had a normal female karyotype (46 XX) The non genital malformations included oesophageal atresia tracheo-oesophageal fistula imperforate anus and renal dysgenesis

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2 Berger G M B & Klempman S A case of female hermaphroditism with associated congenital defects Review of some aetiologic problems. *S Afr Med J 39* 3 1965

3 Butler L J Snodgrass G J A I France N E Russell A & Swain V A J True hermaphroditism or gonadal intersexuality Cytogenetic and gonadal analyses of 5 new examples related to 67 known cases studied cytogenetically *Arch Dis Childh 44* 666 1969

4 Carpenter P J & Potter E L Nuclear sex and genital malformation in 48 cases of renal agenesis with special reference to nonspecific female pseudohermaphroditism *Amer J Obstet Gynec 78* 235 1959

5 Jones H W & Scott W W *Hermaphroditism genital anomalies and related endocrine disorders* Williams & Wilkins Baltimore 1958

6 Reilly W A Hinman F Jr Pichering D E & Crane J T Phallic urethra in female pseudohermaphroditism *Amer J Dis Child 95* 9 1958

7 Wilkins L Jones H W Jr Holman G H & Stempfel R S Jr Masculinization of the female fetus associated with administration of oral and intramuscular progestins during gestation Non adrenal female pseudohermaphroditism *J Clin Endocr 18* 559 1958

8 Wilson J G & Warkany J Malformation in the genito-urinary tract induced by maternal vitamin A deficiency in the rat. *Amer J Anat 83* 357 1948

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Key words Female pseudohermaphroditism multiple congenital anomalies



Fig 1 App arance of extern il genitalia

tissue which ended in a cystic dilatation Testicular tissue was not found

Chromosome analysis in cultures from the p riph eral blood of both the patient and the mother reveal d normal female karyotype 46 XX

DISCUSSION

Androgenic stimulation cannot explain the non genital anomalies in the present case and



Fig 3 Photomicrograph of the corpus cavernosum with centrally situated urethra H E ×30

it is tempting to suggest a common genesis Masculinization of the external genitalia in this and similar cases seems to be associated with certain malform itions i e renal dys genesis or agenesis imperforate anus and anomalies of the lower limbs (2 4 6) Thus in a review of 48 cases of renal agenesis Carpen ter & Potter found that none of 13 female infants had an entirely normal genital tract and 3 of the infants were female pseudoherma phrodites with a phallic urethra (4) On the other hand associated congenital malforma tions are rare and without consistent pattern in true hermaphroditism (3)

Experimentally maternal vitamin A defi ciency in the rat has been shown to induce pseudohermaphroditism in both female and male fetuses and furthermore persistence of the urogenital sinus and renal dysgenesis with retarded formation of the nephrons (8) This observation indicates that non hormona



Fig 2 Photomicrograph of the inguinal node showing renal glomeruli and tubuli with varying degree of differentiation H E ×370

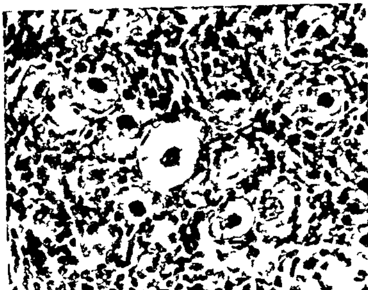


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Acta Pædiat Scand 61 226–229, 1972

THE INTERNATIONAL PAEDIATRIC ASSOCIATION
L'ASSOCIATION INTERNATIONALE DE PEDIATRIE

NEW URBAN FAMILIES

Conclusions and Recommendations of a Workshop on Nutrition[1]
Vienna August 28 1971

Chairmen BO VAHLQUIST THOMAS STAPLETON and MOISÉS BÉHAR

Explanatory note The discussions at the Workshop were restricted specifically to the nutritional problems of families who move from rural to urban areas The phrase New Urban Families has been used to describe them It is clear that the magnitude and the nature of the problems differ markedly in different countries It is also evident that some of the problems relating to their nutrition are generally applicable and that no government can deal with the problems of New Urban Families without also considering the needs of the population of the whole country Nevertheless the problems of New Urban Families are so acute that they merit detailed consideration in their own right

CONCLUSIONS

1 The nutritional state of children in New Urban Families is frequently unsatisfactory

2 A decline both in the proportion of mothers breast feeding their children and in the duration of breast feeding commonly occurs In these circumstances early weaning and inadequate supplementary feeding are important causes of undernutrition during the first year of life

3 Among the factors leading to a decline in breast feeding the following seem to be important

(*a*) Socio cultural changes
(*b*) Mothers going out to work
(*c*) Imitation of friends neighbours or prominent persons who have achieved success in raising their babies with bottle feeding which thus may acquire a higher status value
(*d*) Easy availability of human milk substitutes
(*e*) Inappropriate promotion of commercial infant formulas
(*f*) Misconceptions on the part of mothers about the value of breast milk
(*g*) Personal embarrassment
(*h*) Fear of losing female attractiveness
(*i*) Mothers busy with social engagements
(*j*) Psychological stresses
(*k*) Lack of sympathetic and enthusiastic orientation of health workers including doctors both in and out of hospital
(*l*) Influence of largely disease orientated health services

4 Unavailability of home grown foods higher costs and insufficient understanding of shopping and monetary values in cities contribute to the children in New Urban Families receiving an unbalanced and unsatisfactory diet

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[1] With the co operation of the World Health Organization United Nations Children's Fund Food and Agricultural Organization of the United Nations and the International Federation of Gynecology and Obstetrics

[2] These are not listed in any order of priority

5 Family stresses from economic employ ment and housing difficulties contribute to nutritional hazards

6 The rapid growth of New Urban Areas often lead to shanty towns with an inadequate water supply and poor sanitation with con sequent detrimental effects on nutritional sta tus

7 Advertising by commercial firms may lead to parents buying foods which are not the most suitable or the most nutritious for their children and it often leads to an injudicious use of limited financial resources

8 The use of improperly prepared or exces sively diluted infant foods may lead to nutri tional deficiency

9 Two major lines of attack are necessary

(a) Because of the immense difficulties of providing a substitute for breast milk which *under the prevailing conditions* is even mar ginally acceptable from nutritional hygienic and financial points of view maximal efforts should be made to counteract a further decline in breast feeding

(b) When all attempts to encourage breast feeding are not being successful and cow s milk preparations are not available the use of inexpensive feeding mixtures essentially based on plant foods which can fulfil the needs of growing infants must be considered A small percentage of animal protein enhances the nutritive value of such mixtures using limited supplies of milk and/or fish protein concen trate in this way may be most rewarding

10 Milk provision from outside sources can not especially in the more populous countries meet the nutritional needs of all the children on a secure or permanent basis such supplies being inevitably inadequate and also subject to sudden cut-off because of political or other changes

11 Paediatricians should engage themselves positively and energetically in systematic ef forts to promote the domestic production of protein rich low-cost foods useful as supple ments during the weaning period This can relate both to commercial production and to home made foods The latter deserve much more prestige than has been given to them so far In many areas it is more useful for wean ing foods to be home prepared rather than in- dustrially produced

12 In some areas calorie deficiency may be as important as protein deficiency

13 Foods from indigenous sources must be acceptable to the population be economical have good storing qualities and be available on a permanent basis Problems in acceptability of such foods are often associated with prestige attitudes

14 Every effort must be made to ensure that when milk or other protein rich foods are pro vided from outside sources they do not

(a) depress efforts to increase the production of protein rich foods from indigenous sources

(b) make the acceptance of indigenously avail- able protein rich foods more difficult to achieve

15 Problems of hygiene in the preparation of foods for infants should be emphasized notably the hazards associated with bottle feeding

16 Before making changes careful con sideration should be given to established local practices and the reasons for these

17 Grossly inadequate feeding of institu- tionalized children is unfortunately still a prob lem in some urban areas Sufficient attention should be given to providing and properly controlling the hygiene of nutritious diets

18 Special attention should be given to food supplementation for pregnant and nursing mothers

19 Efforts should be designed to meet the important nutritional needs of the preschool and the out of school school age child

20 Attention should be given to the advice and services to families which can lead to the spacing or limiting of pregnancies this being of great importance not only for the nutrition of the mother but also of the offspring

21 Many situations highlight the necessity for co-operation between paediatricians ob-

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viding solely curative medical services should be emphasized to governments[1]

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[1] The cost for the hospital care of one single child with severe malnutrition may amount to large sums which could be used much more appropriately in preventive health work to the benefit of a number of children

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International Paediatric Association
Institute of Child Health
Royal Alexandra Hospital for Children
Camperdown N S W 2050
Australia

stetricians, nutritionists educators, economists agriculturalists and social authorities at national community and family levels

22 Sometimes there is a lack of understanding by the executive officers of governments of the seriousness of the nutritional problems in New Urban Families

RECOMMENDATIONS

1 The seriousness of the problem of malnutrition in New Urban Families should be brought to the attention of governments

2 Similarly steps should be taken to ensure that the problem be better recognized by medical and paramedical personnel

3 The maintenance of breast feeding is a major public health measure Every effort to promote it should be made through education and by emphasis in both the training of health personnel and in the planning of a food and nutrition policy

4 While recognizing that the maintenance of breast feeding is of the greatest importance action should be taken to minimize the ill effects of any decline in its incidence and duration

5 It must also be recognized that cow's milk whether fresh or processed is often not available nor likely to be available in sufficient quantity for a majority of children in New Urban Families so action must be taken to provide alternative foods from indigenous sources

6 Exchange of information about the problems of preparing and marketing protein rich foods should be encouraged

7 Realizing the paramount importance of food nutrition during early life especially during the first six months and that under the conditions prevailing among under privileged groups in developing countries it is extremely difficult to substitute any suitable food for breast milk the formula producing food companies should observe great caution in applying methods of promoting their products

8 The skills and interests of commercial companies may be used in the promotion and distribution of supplementary foods which are suitable both in composition and cost

9 Persons contacting mothers should be so well informed that they are able to look at the nutritional problems of the family as a whole They should never use their influence to promote a particular product in such a way that it could be detrimental to good breast feeding practices

10 Public educational efforts in nutrition should be strengthened through school programmes mass media etc as well as through family care

11 All opportunities of contact with health services homes clinics and hospitals should be utilized for the nutritional education of children parents and families

12 The paediatrician should feel it is his responsibility as a specialist in child health to engage very actively in the communication of sound knowledge in infant feeding

13 In educational programmes related to the feeding of infants and young children much emphasis should be given to influencing the New Elite groups

14 Examples of research aimed at collecting much needed new information would be

(a) An international co ordinated study on breast feeding in different areas of the world (The socio cultural dynamics of breast feeding)

(b) An international co ordinated study on the nutritional status of New Urban Families

15 Because of the close interrelationship between nutrition infection and the spacing of pregnancies none can be considered in isolation

16 Training programmes should be improved and expanded so that more and better qualified persons are available to provide the necessary education and services to the public

17 The importance of improving the nutritional state of children as compared with pro

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting Jan 13 1971

J Cohn & V Gerhard Nielsen *Development of inhibitor to factor VIII in patients with haemophilia A*

Four cases of haemophilia A complicated by development of inhibitor to factor VIII are presented

The method of determining the inhibitor is described Investigation of the patients for this factor is recommended when the diagnosis of haemophilia A is established prior to operative intervention and when there is evidence that treatment with AHG is inadequate and even possibly before every treatment with AHG is commenced

If the inhibitor is present treatment of severe haemorrhage consists of administration of very concentrated factor VIII preparations possibly glucocorticoids and immunosuppressive preparations and erythrocyte suspensions with low leucocyte content in order not to stimulate antibody production further

Vibeke Faurholt Pedersen *A case of methyl malonic acidosis*

The patient a girl was admitted at the age of 5 weeks to the Department of Paediatrics in Hillerød on account of failure to thrive She was the second of two siblings and her older sister had died at the age of 6 months from a similar clinical condition Investigation of the urinary content of amino-acids in the sister had revealed a relatively high content of glycin and autopsy revealed fatty degeneration in the liver The family history did not reveal any other diseases

The pregnancy and delivery in the present patient had been normal and the birth weight was 2 950 g On admission she showed a greyish pallor was miserable and moderate metabolic acidosis was present The weight was 3 260 g She vomited repeatedly and massive constipation was present

Radiographic investigation of the alimentary canal did not reveal any abnormalities Cerebral symptoms became increasingly evident torpor hypotonia fine tremor exaggerated reflexes and nystagmus The EEG showed severe diffuse abnormalities Hepatomegaly anaemia thrombocytopenia and neutropenia developed The diagnostic deliberations were concentrated on a congenital metabolic condition Reproducible increase in the glycin content in the blood and urine was compatible with the diagnosis of hyperglycinaemia To establish the differential diagnosis the urine was investigated for methyl malonic acid (MMA) and the excretion was found to be grossly increased 3 000 mg as compared with the normal maximum value of 10 mg per 24 hours The serum vitamin B_{12} level was normal

The patient was treated with a diet with a fixed protein level of 2 g per kg/24 hours in the form of breast milk with supplementary iron vitamins and $NaHCO_3$ and rapidly became clinically symptom free As a single injection of vitamin B_{12} resulted in a prompt fall in MMA excretion to 300 mg/24 hours the treatment was supplemented with 30 μg

PROCEEDINGS OF PAEDIATRIC SOCIETIES

FINNISH PAEDIATRIC SOCIETY

Meeting May 19, 1971

John Money (Baltimore, USA) *Assignment of sex and psychological management of intersexual children in light of long term experience*

Meeting May 22, 1971

P Gronroos (Tampere, Finland) *Computer experience from the central hospital of Tampere*

R S Ikonen (Tampere, Finland) *The Apgar score evaluation and asphyxia of the newborn* Before Apgar, in 1953 published her now widely spread scoring system, there was no uniform basis to evaluate the asphyxia and the general status of the newborn Several studies have thereafter shown that there is a clear correlation between the Apgar score estimated at the age of 1 minute and 5 minutes and the neonatal mortality

The infants given a low score at the age of 1 minute may be divided into the following groups 1) fetal asphyctics 2) those under influence of analgesics or anesthesia 3) those with a trauma from delivery and 4) infants who have some disease causing poor condition immediately after delivery A great deal of the cases show combinations of the above groups thus complicating the picture of the causes of low scoring

The drawbacks of the Apgar scoring are e g 1) It reflects the status of the newborn only at a certain moment 2) it ascribes the same value to all the components of the score estimation, such as the heart rate respiration etc, which surely does not hold true 3) it can not distinguish the primary and secondary apneas from each other, which would be very important in determining the treatment of an asphyctic infant, 4) the significance of the Apgar score may be over rated so that the observation of a high score infant is neglected in assuming that it cannot have any more trouble

The advantages of the Apgar scoring may be considered to be 1) The status of a newborn is paid attention to 2) the score estimation gives an indication of the need of immediate treatment and its method 3) it also reveals the group of infants requiring special attention i e all infants with a low score, 4) it intensifies the co operation of obstetricians and pediatricians and 5) it gives a more uniform basis for studies concerning e g asphyxia

Olli Koskimies

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and later 1 000 μg Cycobemin intramuscularly once weekly Follow up examination at the age of 9 months revealed normal psychomotor development for her age The EEG and amino acid excretion had returned to normal

Minna Yssing *Paroxysmal familial choreo athetosis*

Bengt Zachau Christiansen *Paediatrics in Greenland*

Meeting Febr 11, 1971

Peter Bækgaard *A case of spondylitis*

The case history of a boy aged 6 years suffering from pyrexia poor general condition flexion contracture in the hip, diffuse abdominal symptoms meningism and back pain is presented The ESR was raised and leucocytosis was present while the other relevant blood and serum tests were negative Radiography revealed narrowing of a lumbar intervertebral space with irregularity of the adjacent vertebral bodies in the third week of the disease Regenerative changes with sclerosing and smoothing became apparent in the third month

The patient recovered following antibiotic treatment after demonstration of staphylococcus aureus from the infective focus in the spine

The clinical picture has been described in the literature under various names discitis juvenile spondylitis, pyogenic osteomyelitis and non specific spondylitis of childhood It may occur either in a pyogenic form with a maximum age of about eight years or in a benign juvenile form without infective cause which frequently occurs about the ages of four to five years The course of the latter form is milder and has fewer sequelae in the form of anchylosis and abscess formation A history of preceding trauma to the back is frequently obtained

Discussion

P H Bræstrup Did blood culture reveal anything?

B Friis Hansen How did the Moro reaction react?

P Bækgaard Both of these investigations yielded negative results

Erik Thamdrup Quoted a similar case from the Paediatric Department in the Central Hospital in Hillerød

Marianne Hertz *Incontinentia pigmenti*

Incontinentia pigmenti (I P) is a familial skin disease which is frequently accompanied by changes in other ectodermal tissues The skin changes develop shortly after birth practically exclusively in girls and undergo an acute inflammatory vesicular stage with eosinophilia and end with peculiar greyish pigmentation which was described for the first time by Bloch and Sulgberger in 1926 and 1928

Twenty per cent of the patients with IP have uncharacteristic CNS changes in the form of spasticity, epilepsy and microcephaly Approximately 33% have ocular changes and nearly half of these consist of tumour bulbi which is recognized, as a rule about the age of one year and which is difficult to differentiate from glioma in the later scarred stages Inflammatory changes have been demonstrated in the early stages Thirty three per cent of the patients with I P have dental defects in the form of hypoplasia and aplasia

On the basis of 215 cases (210 girls and six boys) Lenz concluded that there is probably a sex-linked disease in the X chromosomes which is fatal in hemizygotes In order to explain the combination of heredity permanent defects and acute transient neonatal skin inflammation Burmeister suggested heredity + sensitization (cf eosinophilia) analogous to erythroblastosis The antigen and details of the mechanism are not yet elucidated

Case history A newly born female infant was admitted on account of a vesico papular skin eruption The general health appeared to

be unaffected The only abnormal finding was eosinophilia of 40 / in the blood The WR and herpes complement fixation reaction were negative Ophthalmological examination and radiography of the teeth did not reveal any abnormalities Skin biopsy revealed findings characteristic of I P At the age of 3 months the child was normally developed but had isolated patches of pigment The mother had presented a similar clinical picture in infancy and had been described by Haxhausen & Heilesen At the age of 8 months one eye had been enucleated after scarring and shrinkage Now at the age of 27 years she has inconspicuous patches of pigment and some teeth are absent

J Haahr & B Halveg *Congenital Leukaemia* Published in *Acta Pædiat Scand 60* 720 1971

J Haahr & T Marner *Complications of umbilical venous infusions in infants*

Infusion via the umbilical vein to ill neonates requiring parenteral therapy has been employed increasingly frequently in many paediatric departments It is easy to introduce a catheter and the method permits long term infusion Only few accounts of complications of this form of therapy have been published and the authors are interested to hear of others

During the past 3 years umbilical venous infusion has been employed routinely in ill neonates particularly in infants with the respiratory distress syndrome in the paediatric department Glostrup Hospital The material comprizes 230 infants of whom 91 (40 %) died during the neonatal period Twelve complications in 10 infants were demonstrated (4 6 %) including 3 cases of thrombosis in the umbilical vein 2 cases of damage to the hepatic parenchyma 2 cases of sepsis 1 case of severe haemorrhage and 4 infants collapsed in connection with introduction of the catheter Only one infant weighing 1 300 g died a few hours after catheterization

The incidence of complications in this material is slight but the investigation gives no information about the subsequent risk of portal hypertension and parenchymatous liver damage

Infusion into the umbilical vein is therefore recommended only for very ill infants and the duration of the treatment should be limited The catheter should be soft and its localization should be confirmed by radiographic control

Karen Thomsen & Stig Sparrevohn *Schönlein Henoch s Purpura*

N J Brandt

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC ASSOCIATION

Meeting May 19 1971

J K Visakorpi *Latent coeliac disease 'Early diagnosis is possible and important*

H Kollberg *Cystic fibrosis*

T Berg *Congenital immune deficiencies*

T Foucard *Asthma bronchiale*

O Hansson *Myasthenia gravis*

O Westphal *Hypothyroidism*

T Tuvemo *Hypoparathyroidism*

S Sjolin *Wilson s disease*

K-H Gustavsson *Congenital adrenal cortical hyperplasia*

These communications will be published in Lakartidningen'

L Hambraeus & O Westphal *Aspects on the diagnosis of ketotic hypoglycemia*

Ketotic hypoglycemia was described in detail in 1964 by Colle & Ulstrom and is characterised by attacks of hypoglycemia when the patients are given a ketotic diet containing 75% of fat, 15% of protein and 10% of carbohydrates According to the original description of the provocation test the patients should be given the diet in an amount equivalent to about 700 kcal per m of body area

The authors have studied the effect of the administration of a ketotic diet in 8 cases of spontaneous hypoglycemia and in 2 normal individuals When the diet was given according to Colle & Ulstrom a marked decrease in blood sugar to values below 50 mg/100 ml and a ketoacidosis was observed in all cases Furthermore changes in the plasma levels of several amino acids were observed and thes differences were most pronounced with respect to the branched chain amino acids, leucine, isoleucine and valine These findings are in agreement with the changes in the plasma aminogram which Felig et al recently reported in diabetic ketoacidosis

When the ketotic diet was more adequate in calories (1 750 kcal per m^2 of body area) hypoglycemia was provoked giving values of less than 30 mg/100 ml, marked ketoacidosis as well as a pathologic plasma aminogram only in 3 cases These patients also showed clinical and other biochemical signs of having ketotic hypoglycemia In the remaining patients the blood glucose never reached levels below 50 mg/100 ml, the ketoacidosis was less pronounced and so were the alterations in the concentration of the plasma amino acids In conclusion it was found that a ketotic diet *ad modum* Colle & Ulstrom adequate in calories, facilitates the differentiation of ketotic hypoglycemia from other types of spontaneous hypoglycemia

J Gentz

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN SOCIETY OF PAEDIATRIC PATHOLOGY

Meeting in Helsinki Finland June 10–12 1971

Frøydis Langmark H Sommerschild & K Maurseth (Oslo) *Congenital hepatic fibrosis*

The two first cases of congenital hepatic fibrosis reported in Norway were presented Case 1 Boy 9 years old with complaints of dyspnoea for one year Enlarged firm liver and enlarged spleen were found No special illness earlier no family history Liver function tests, portal venous pressure and esophagram were normal IVP showed enlarged kidneys with normal configurations Renal function tests were normal Explorative laparotomy and biopsy from the liver confirmed the diagnosis of congenital hepatic fibrosis The patient is *doing well* Case 2 Girl 14 years old with massive hematemesis at 10 years of age Portal hypertension and esophageal varices were demonstrated Liver function tests were normal The portal vein was patent by splenoportography Splenectomy and splenorenal anastomosis were carried out Liver biopsy revealed a probable biliary cirrhosis Except for episodes of pyuria during the first two years of life her case history had been uneventful until hematemesis occurred During a 4 year follow up the patient was doing well in spite of persistent esophageal varices At 14 years of age she had a second relatively small hematemesis A review of the liver biopsy specimen supported the diagnosis of congenital hepatic fibrosis IVP showed enlarged kidneys with cystic disease Renal function tests and liver function tests were normal A portocaval shunt was done the postoperative course was unremarkable

150 cases previously reported in the literature were reviewed 75 of these with a detailed case history Hematemesis and/or enlarged liver are the presenting symptoms in 75% most frequently at the pre school age Half of the cases are sporadic one third of these have renal changes mostly of the "tubular ectasia type The remaining half of the cases are familial with affected siblings 70% of these have renal involvement mostly resembling cystic disease of the kidney Hitherto 50 % of all the cases of CHF have died chiefly from uremia massive esophageal hemorrhage or postoperative complications The patients without renal failure and with portal hypertension but with patent portal vein and preserved liver function are ideal candidates for portosystemic shunt possibly also prophylactic

The characteristic histological picture is that of preserved lobular liver parenchyma interspersed with broad fibrous strand of mature connective tissue containing a large number of bile ducts The bile ducts are small or moderately dilated most of them empty The liver cells are normal and there are usually no signs of inflammation Several



Fig 1 Case I Continuous strands of periportal connective tissue surrounding the lobules Increased number of bile ducts Gomori × 50

authors have reported hypoplasia and distortion of the smaller portal vein radicals in the increased periportal areas Macroscopically the liver is enlarged and extremely firm Additional anomalies in other organs have been found in some of the cases (Figs 1 2)

Bengt Larsson (Norrköping) *Postmortem examination of a case with juvenile lipogranulomatosis (Farber)*

Björn I Ivemark (Stockholm) *Lipid histochemical findings in juvenile lipogranulomatosis (Farber)*

Deep frozen specimens from a 16-year old boy were fixed in calcium formalin and sectioned on a thermo electric microtome (PELCOOL MSE) The results were compared with model smears containing the crystalline ceramide isolated from the granulomas by Karin Samuelsson and Rolf Zetterström The granulo-



Fig 2 Case II Increased periportal connective tissue with many bile ducts some of them intralobularly located H erythrosin saffron × 50

Table 1 *Small intestinal biopsy findings in patients with the malabsorption syndrome induced by cow s milk*

	Initial biopsy					Biopsy after treatment				
Group	Total	Norm	Slight changes	PVA	SVA[b]	Total	Norm	Slight changes	PVA	SVA
I	18	1	1	8	8	20	18	0	2	0
II	23	0	1	6	16	23	13	2	7	1

PVA = partial villous atrophy [b] SVA = subtotal villous atrophy

mas contained cells of two types PAS-negative and PAS-positive The PAS-negative showed large doubly refractile crystals with the *same tinctorial properties at the pure cer*amide The PAS-positive cells did not contain ceramide but a non refractile glycolipid easily removed by chloroform methanol extraction and also positive in the modified PAS-reaction as given by Adams This second substance might be ganglioside Thus the juvenile *form of Farber s disease shows* accumulation in granulomas of two different substances—ceramide and a glycolipid possibly ganglioside The kidneys contained ceramide in tubular cells and also in PAS-positive substance with staining reactions similar to those of the granulomas Samuelsson & Zetterstrom have shown that the *renal ceramide* is mainly composed of alpha hydroxy fatty acids while that of the granulomas chiefly contains palmitic acid However it was not possible histochemically to differentiate between the ceramide found in the kidney and that of the granulomas The enzymatic defect in this disease remains to be elucidated

H Søgaard & Sv Bittmann (Aarhus) *A branchial facial malformation*

A one month-old girl had in her left zygomatical region a 18 × 6 × 6 *mm soft* elastic process coated with normal skin but with a central *firm chord*

She was child no 2 no congenital malformations were observed in the family pregnancy and delivery normal. The mother had received no drugs during pregnancy

The child was operated on at 1 month of age

The microscopical examination revealed a nearly perfect symmetrical skin dowel with an axis consisting of nerves and vessels surrounded by longitudinal streaks of striated muscle The muscle had no connection with the facial musculature developed from the sec*ond branchial arch and the central tendon* was fixed to the centre of os zygomaticum The malformation might be an excrescense of the axis of the maxillary branchial arch developed rather late in fetal life and without relation to the previously *reported cases of* congenital teratomas arising from the celfts *in the skull and fusions of the* branchial arches

P Kuitunen J Rapola E Savilahti & J K Visakorpi (Helsinki) *Light and electronmicroscopic changes in the small intestinal mucosa in patients with cow s milk induced malabsorption syndrome*

The *series consists of* 47 infants seen during 1963–1970 It was divided in two subgroups group I 22 patients who had never received gluten and group II 25 patients who had received gluten before admission The division was made to extract possible coeliac *patients* out of this series The patients reacted to cow s milk with vomiting diarrhea and weight loss Malabsorption was found in most of the patients *The* villous structure of the jejunum was damaged in most of the cases but the lesion was however more severe in the group II than in the group I (Table 1)



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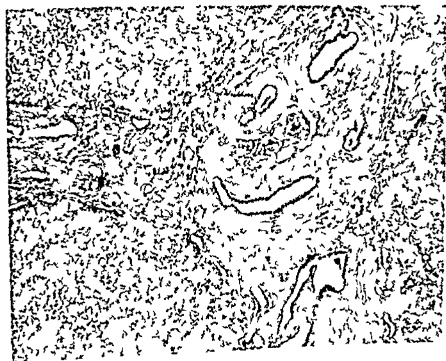


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Fig 3 Electron microscopic appearance of air expanded rat lung fixed by immersion in glutaraldehyde The smoothly curved surface of the alveolar lining layer indicates preservation of the air-liquid interface during fixation Part of the interface is formed by a "lattice figure" (*arrow*) Lead citrate uranyl acetate × 60 000

Focal non specific ulcerations may be found in the vermiform appendix in cases of enteric infection with *Yersinia enterocolitica* There sometimes is a large pyroninophilic cell reaction in the cortical pulp of mesenteric lymph nodes The large pyroninophilic cell reaction was associated with an early production of specific antibodies The immunological and diagnostic aspects of the large pyroninophilic cell reaction were discussed and some data were given on its occurrence in other infections and immune reactions The studies have been published in *Acta Path Microbiol Scand* Section A *79* 109 1971

Jan Modee & Bengt Robertson (Stockholm)
Fixation of the alveolar lining layer with preservation of the air–liquid interface

Demonstration of the alveolar lining layer by electron microscopy requires preservation of the air–liquid interface during fixation This is easily achieved with the following technique When the thorax of the experimental animal is opened the lungs are kept air expanded under an endotracheal pressure of 10 cm H O This pressure is maintained as the lungs are fixed by immersion in 2% glutaraldehyde Tissue blocks for electron microscopy are then cut from the periphery of the lungs The further procedure includes osmication in 1% osmium tetroxide dehydration in acetone and embedding in Vestopal W When examined with the electron microscope the alveolar lining layer displays a smoothly curved surface (Fig 3) The electron density of the alveolar lining layer its mucopolysaccharide component in particular is increased if ruthenium red (1 mg/ml) is added to the fixative and to the osmium solution

H Søgaard & Sv A Askjær (Aarhus)
Pseudomembranous enterocolitis in megacolon congenitum (Hirschsprung)

Case history 5 year old boy typical mongoloid appearance Since birth chronic obstipation caused by megacolon Admitted to a hospital for acute gastro-enteritis died the first day of cardiac arrest

Post mortem Severe bilateral lobar pneumonia with greyish red tissue The microscopical examinations showed lipid pneumonia with hyperplasia of the alveolar epithelium and giant cells without nuclear inclusion bodies

The jejunum and in a lesser degree the ileum showed a severe membranous enteritis and similar changes were found in colon in addition to the typical Hirschsprung megacolon and fibrous non specific colitis The mucosa in the intestine was necrotic but there were only very few granulocytes

A lipid pneumonia of several days duration had weakened the resistance of this child and

The patients were placed on the elimination diet (breast milk and/or soy milk) until they again could tolerate cow's milk. The normalization of the intestinal mucosa was evident (Table 1). After the mucosa had become normal the patients were allowed to eat ordinary food. Follow up biopsies after one year indicated that 11 out of 18 patients in group I and 4 out of 11 in group II had normal villous structure. As in coeliacs administration of free diet for long periods in most cases results in villous atrophy, we believe the healing in these cases is permanent. A patient with SVA in the group II is suffering from coeliac disease. The follow up time for the patients with PVA in both groups is too short to make final conclusions.

Electron microscopic studies was done in one infant with malabsorption caused by cow's milk. In the active stage of the disease the microvilli were short and often branched and fused. The mitochondria and the nuclei of the epithelial cells showed degenerative changes. During the elimination diet with breast milk these changes became much slighter. The provocation with cow's milk made these changes worse and the number of lysosomes in the upper part of the epithelial cells increased clearly.

This study shows that the small intestinal damage in cow's milk induced malabsorption syndrome is a transient phenomenon but some of these patients are real coeliacs. At the ultrastructural level there are changes which partly resemble those found in coeliac disease.

Erkki Savilahti (Helsinki). *Local immunological reaction in the small intestinal mucosa in children with the malabsorption syndrome induced by cow's milk*

The local immunological reaction of the small intestine to cow's milk was studied in 10 children with an established, diagnosis of malabsorption induced by cow's milk. The number of immunoglobulin containing cells in the small intestinal mucosa was estimated at various stages of their disease by the direct immunofluorescent technique.

In the primary biopsy specimens of 2 cases the numbers of IgA- and IgM-containing cells clearly exceeded the corresponding numbers in an age matched control group.

After an elimination diet (breast milk) for variable periods, the patients were again exposed to cow's milk. At this later exposure 7 of the 10 had a clinical relapse, while 3 already tolerated cow's milk.

At the beginning of this exposure the numbers of IgA and IgM-containing cells were in the normal range. After the clinical reaction, which took place after a period varying from a few hours to 8 weeks, the average number of IgA containing cells was 2.3 times as high as the pre exposure value. In the 3 patients who were clinically tolerant of cow's milk the rise in the number of cells was less marked, mean 1.7. The number of IgM-containing cells changed in a similar manner, the average rises being 2.3 and 1.7 fold in the respective groups.

Two children with unspecific malabsorption syndrome was followed during a similar change of diet from breast milk to cow's milk. In these cases no constant changes in the numbers of IgA- and IgM containing cells were observed. The study shows that children with the malabsorption syndrome induced by cow's milk, when re exposed to this food react with a strong elevation of IgA and IgM containing cells in the small intestinal mucosa. As a similar elevation is observed in the initial phase of the disease, this could be an important factor in the pathogenesis of the syndrome.

J. Ahlqvist, P. Ahvonen, J. A. Rasanen & G. R. Wallgren (Helsinki). *Enteric infection with Yersinia enterocolitica. Large pyroninophilic cell reaction in mesenteric lymph nodes associated with early production of specific antibodies*



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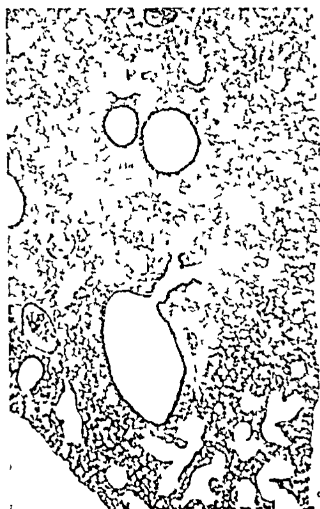
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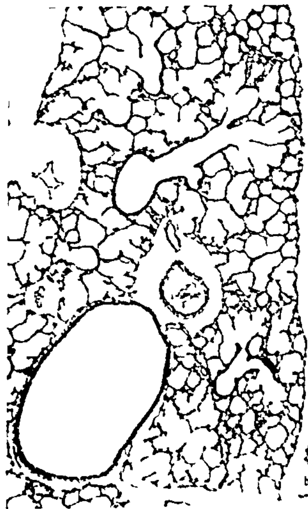


Fig. 4. Air-expansion patterns in NaCl treated control fetus (*A*) and in surfactant treated fetus (*B*). The lungs were fixed at endotracheal deflation pressure of 10 cm H_2O. Hematoxylin & Eosin. ×33.

caused a pseudomembranous enterocolitis probably of viral genesis. The connection between megacolon, chronic colitis and megacolon congenitum (Hirschsprung) was discussed and documented by photos.

Göran Enhörning & Bengt Robertson (Stockholm): *Improved air expansion of the premature rabbit lung after tracheal deposition of surfactant*

The air expansion of the lungs of premature rabbit fetuses (gestational age 28 days) was improved by tracheal deposition of a concentrated surfactant suspension obtained by centrifugation of alveolar wash from an adult rabbit for 1 h at 1 000 *g* and 4°C. The beneficial effect of surfactant deposition was apparent in pressure-volume recordings as well as in histological lung sections from the experimental animals (Fig. 4). Our results suggest that tracheal deposition of surfactant before the first breath might be adopted as prophylaxis against the idiopathic respiratory distress syndrome, which is known to be related to surfactant deficiency in the pulmonary fluid. Infants at risk might be identified by phospholipid analysis of amniotic fluid obtained by amniocentesis according to the methods recently described by Gluck et al.

Reference

Gluck, L., Kulovich, M. V., Borer, R. C. Jr, Brenner, P. H., Anderson, G. G. & Spellacy, W. N.: Diagnosis of the respiratory distress syndrome by amniocentesis. *Amer J Obstet Gynec 109*: 440, 1971.

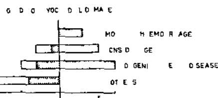


Fig 5 Autopsy material divided into different disease class s Horizontal lines severe myocardial damage crossed some myocardial damage white no damage as judged from fuchsinophilia

Erkki Pesonen (Helsinki) *Myocardial ischemic damage in the newborn*

Acidosis hypoxemia and hypoperfusion of organs are commonly encountered during the terminal stage of several neonatal disorders This shock condition causes various organ manifestations Myocardial damage was studied in 32 consecutive autopsies of patients under 2 months of age Samples of heart muscle were taken from six different sites Fuchsinophilic staining of the cells served as a sensitive index of myocardial damage (1)

The most extensive changes were seen in children who had died of severe congenital heart disease Myocardial damage was also found in children whose death was caused by CNS disorders In all three cases of massive pulmonary hemorrhage there were extensive changes Patients who had died primarily of immaturity hyaline membrane disease or infection did not show appreciable myocardial damage (Fig 5) In grave congenital heart diseases (hypoplastic left heart syndrome double outlet right ventricle transposition of the great arteries) an anatomically abnormal heart has to perform heavy work under hypoxic conditions and will therefore sustain injury Coronary perfusion is too small to satisfy the oxygen need The ischemic changes in cases of massive pulmonary hemorrhage suggest that myocardial damage causes bleeding It is not clear why there is such extensive myocardial damage in the group dying of CNS disorders One speculative possibility is pathological liberation of catecholamines

Reference

1 Connor R C R Th demonstration of rec nt myocardial injury A simple method suitable for routine use *J Pa h Bact 101* 71 1970

Matti Haltia Juhani Rapola & Pirkko Santavuori (Helsinki) *Neuronal ceroid lipofuscinosis of early onset A report of 6 cases*

Recently 6 cases of neuronal ceroid lipofuscinosis (1) of exceptionally early onset were seen at the Children s Hospital of Helsinki Five of them were diagnosed by brain biopsy one case was detected at autopsy

The age of the patients at the time of the biopsy varied from 1 year 8 months to 4 years The autopsy case was a boy of 6 years 10 months The patients appeared to develop normally until 6–12 months of age when psychomotor retardation was noted Hypotonia and ataxia were soon observed rapidly followed by myoclonic jerks tremor rigidity spasticity amaurosis and dementia Electroencephalography revealed severe non specific abnormalities Electroretinography was performed in 4 cases no activity could be demonstrated Pneumoencephalography showed generalized atrophy Vacuolized lymphocytes could not be found

Examination of the biopsy and autopsy specimens revealed similar changes in all cases The scanty cytoplasm of the cortical neurones was distended by coarsely granular autoflourescent sudanophilic PAS positive acid fast material The material was not extracted with chloroform methanol There was severe neuronal loss and in the older cases only extremely few neurones remained In all cases the cortex was studded with large macrophages containing similar material as in the nerve cell bodies The astrocytes even those of the white matter showed lesser amounts of this material Considerable myelin loss was seen particularly in the older cases accompanied by heavy astrogliosis Biochemical analyses of the ganglioside pattern revealed only minor probably non specific abnormalities in the older cases

On the basis of these findings the cases may be classified as ceroid lipofuscinosis They differ from the previously recognized types within this group by the very early onset, rapid course, reflected by the severely destructive nature of the histological changes, and absence of vacuolized lymphocytes

Reference

1 Zeman W & Dyken P Neuronal ceroid lipofuscinosis (Batten s disease) Relationship to amaurotic family idiocy? *Pediatrics* *44* 570 1969

Juhani Rapola & Jussi Vilska (Helsinki) *Kidney structure in the congenital nephrotic syndrome*

The congenital nephrotic syndrome (CN) is a familial disorder characterized by onset in the first few weeks of life and shows resistance to all kinds of treatment and leads to early death It is a rare condition its prevalence being highest among the Finns We made microscopic analyses of the kidneys of 43 typical CN patients from 3 weeks to 2 years of age The material consisted of both premortem biopsies and material obtained at autopsy

Glomerular changes could be classified into three categories 1) Immature glomeruli with a continuous layer of visceral epithelial cells covering the capillary tufts with or without widening of the urinary space 2) Glomeruli showing mesangial cell proliferation with accumulation of a PAS- and silver positive fibrillar matrix in the mesangium Most of these glomeruli had very wide peripheral capillary lumens 3) Glomeruli in different stages of total capillary obstruction and sclerosis In quantitative counting the proportion of immature glomeruli decreased whereas the mesangioproliferative and obstructed glomeruli increased with the progression of the disease The proportion of obstructed glomeruli however, never exceeded 20% and was usually much less Except for some focal areas no basement membrane thickening was found

Microcytic dilatation of proximal and distal tubules was observed in part of the material It was most prominent in the specimens from the oldest patients and those from many of the younger patients showed normal or very slightly dilated tubules Interstitial lymphoid cell proliferation accompanied the microcystic change of the tubules

In nearly all specimens the walls of the cortical small arteries and arterioles showed medial and intimal thickening

Although the pathological changes in each individual varied the pattern of changes was rather uniform We suggest that the criteria outlined above should be used in the histological diagnosis of specimens from a patient suspected to have CN of the Finnish type

Biorn Ivemark

Acta Pædiat Scand 61 243–270 1972

PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

Meetıng ın Zurıch May 8–12 1971

D Knorr (Munchen) *Synthesıs and metabolısm of testosterone*

Cholesterol ıs an essentıal precursor of the steroıd hormones both ın the adrenal glands and ın the gonads These glands have the abılıty to buıld up cholesterol from acetate The synthesıs of cholesterol by the gonads ıs certaınly dependent on the gonadotrophıns LH or HCG

In the adrenal glands and ın the gonads the cholesterol sıde chaın ıs splıt off after hydroxylatıon at C $_0$ and C forming Δ pregnenolone The tıme sequence of the next enzymatıc steps ıs not known wıth certaınty Two metabolıc pathways are well recognızed the Δ^4 pathway through progesterone and the Δ^5 pathway through DN Androstenedıone ıs the end product of both pathways Androstenedıone ıs converted ınto testosterone by a 17 dehydrogenase Some recent research wıth labelled substrates show however another ımportant metabolıc pathway whıch by passıng androstenedıone leads dırectly from DHA to testosterone

Some workers could not fınd the expected fıssıon product ısocaproıc acıd from the splıttıng off of the cholesterol sıde chaın by the testıcular tıssue Two groups on the contrary ın such ınvestıgatıons recently dıscovered methyl heptanone as a fıssıon product Thıs fındıng poınts to a completely new metabolıc pathway from cholesterol dırectly to the C_{19} steroıds by passıng the C_{21} steroıds

Eık Nes showed ın dogs testıs perfusıon studıes that as lıttle as 10 I U HCG ınjected ınto the spermatıc artery wıthın 20 sec was suffıcıent to ıncrease the testosterone concentratıon ın the spermatıc veın by 60 ′ There was a marked ıncrease of testosterone androstenedıone and DHA only from the sıde ın whıch the HCG was ınjected

Testosterone secretıon can be stımulated ın chıldren of all ages The admınıstratıon of 1 500 I U HCG every second day to boys of all ages ıncreases the plasma level of testosterone to that of an adult man wıthın 2 weeks

The ıntratestıcular metabolısm of testosterone before puberty ıs not the same as after puberty Steınberger showed that ın the rat the adult testıs metabolıses progesterone to testosterone but the prepubertal testıs to androstandıol

In males the productıon rate and the urınary excretıon rate for testosterone ıs ın the same range In females the urınary excretıon rate of testosterone ıs about three tımes as hıgh as the productıon rate ındıcatıng a perıpheral ınterconversıon The mean precursor for the perıpheral ınter-conversıon to testosterone glucuronıde ıs androstenedıone About 1 / of the secreted testosterone ıs excreted as testosterone glucuronıde In the target organs for ınstance ın the prostate gland testosterone ıs assocıated wıth cell nucleı and reduced to 5α-dıhydrotestosterone In pregnancy the plasma testosterone level ın the mother ıs hıgh In spıte of these hıgh testosterone levels the mother ıs protected from vırılısatıon by a hıgh testosterone bındıng globulın level There ıs no dıfference ın plasma testosterone levels

On the basis of these findings the cases may be classified as ceroid lipofuscinosis They differ from the previously recognized types within this group by the very early onset rapid course reflected by the severely destructive nature of the histological changes and absence of vacuolized lymphocytes

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dehydrogenase deficiency as reported previously And although Δ^5 pregnene 3β 16α diol 20-one was often high in the first days of life in the former there was considerably less in the latter—a matter not previously recognized and of uncertain significance

It is proposed that the maturation of an hepatic 3β hydroxysteroid dehydrogenase (and isomerase) is responsible for the peripheral conversion of Δ^5 3β hydroxysteroids The oral administration of Δ pregnene 3β 17β-dihydroxy 20-one to normal volunteers led to the excretion of pregnanetriol exclusively without any evidence of precursors with the Δ 3β hydroxy grouping Such a complete conversion following oral administration can hardly be attributed to an adrenal enzyme Studies are underway with the livers of fetal newborn and mature experimental animals to substantiate this thesis

R Rappaport & J M Limal (Paris) *Evaluation of gonadal and adrenal activity at the initial stages of puberty*

Recent progress in the investigation of puberty in man has been made possible by careful longitudinal studies of physical development and by more sensitive and reliable biochemical techniques A tentative correlation between clinical events and hormonal variations will be presented for precocious pubarche and thelarche gynecomastia and growth spurt

During early puberty the adrenal cortex undergoes maturation and secretes dehydroepiandrosterone and its sulfate For both hormones plasma levels are increasing More indirectly this maturation has been expressed by an increase in 11-deoxy 17 ketosteroid urinary excretion (mainly DHA and DHAS) Plasma testosterone measurable in prepubescent boys and girls increases slowly with important individual variations Testosterone metabolites contribute to urinary 11-deoxy 17 ketosteroids (androsterone being fairly significant at the initial stage of puberty only to a small extent Hepatic conversion from androstenedione and DHA is probably minimal but in girls this fraction should be remembered in the discussion of plasma testosterone and urinary glucuronide of testosterone principally Ovarian function study has been limited to routine measurement of urinary estrogens and exfoliated vaginal cells Recent competitive binding techniques or radioimmunoassay should offer new data to correlate with already available cross sectional studies of plasma testosterone LH and FSH Peripheral metabolism of androgens plays a major role in skin and others androgen receptors 5α reductase activity allows conversion of testosterone to dihydrotestosterone which is one of the active intracellular forms of androgens On the other hand plasma testosterone binding affinity decreases during puberty in both sexes This could be the result of increasing levels of gonadal and adrenal androgens and it may eventually play a role in the modification of the 5α reductase activity

In normal puberty pubic hair stage 2 may precede breast development in girls and testicular enlargement in boys It is absent in cases with combined deficiency of LH and ACTH secretion present although scanty in cases with gonadal agenesis and castrated subjects Such situations favor a prominent role for adrenal androgens in pubic hair development

Precocious pubarche is a particular stage of partial puberty in girls and is less frequent in boys Bone age height and growth rate are normal or slightly advanced while breasts and (or) testes are prepubescent Hormonal changes when present reflect premature adrenal maturation increase of DHA and DHAS normal androstenedione adult female levels of testosterone in plasma slight increase of urinary testosterone glucuronide and suppressible by dexamethasone Plasma LH and FSH have been reported to be normal or even low Such a hormonal pattern does not provide argument for an unique mechanism 1) Premature activation of central control (unknown corticotropic factor different from ACTH and

between mothers carrying male and female foetuses

There are some discrepancies in plasma testosterone levels found before puberty While Frasier reported mean plasma testosterone levels of 53 ng/100 ml in boys and 39 ng/100 ml in girls before puberty we could never detect more than 11 ng/100 ml plasma testosterone before puberty Plasma testosterone levels found in boys and girls show no statistical difference

During puberty the mean testosterone plasma level at the age of about 12 years rises distinctly above the prepubertal value and by 13 years attains the level of 35 ng per 100 ml (normal for adult female) Over 15 years the lower limit for normal men is exceeded and after the 18th year the curve reaches the mean level of 530 ng/100 ml (normal for adult male) Yet this curve conveys a completely wrong idea of the individual course of puberty

In 18 longitudinal studies by Knorr the testosterone levels reached the lower normal male limit at most within 1 year after it exceeded the average level for women in all subjects The decisive increase in plasma testosterone in boys during puberty occurred therefore within a year sometimes even within half a year

A M Bongiovanni (Philadelphia) *Another look at congenital adrenal hyperplasia due to 3β hydroxysteroid dehydrogenase deficiency*

In the original description of congenital adrenal hyperplasia due to 3β hydroxysteroid dehydrogenase deficiency it appeared that in the urine Δ pregnenetriol predominated and that pregnanetriol was rarely present although it was described in one instance This was attributed to a double enzyme defect With the passage of time other cases especially those beyond the age of infancy have had pregnanetriol present Therefore the first thesis that this might be due to a double enzyme defect now appears unlikely Furthermore in the usual 21 hydroxylase deficiency, the urine collected in the first few days of life may show substantial quantities of Δ^5 3β hydroxysteroids a feature which is characteristic of many normal infants Confusion may arise in the differentiation between these two types For this reason a re examination was carried out on several cases of both types

Ten cases of 21 hydroxylase deficiency three of 3β hydroxysteroid dehydrogenase deficiency five normals and two of adrenal tumor were studied Extracts of urine were hydrolysed first by the enzyme glucuronidase and then by solvolysis After preliminary column purification the residues were analysed by gas chromatography The trimethylsilyl esters were chromatographed on QF1 at 210°C and 12 psi nitrogen

In early life cases of 21 hydroxylase deficiency particularly in the first week often showed only traces of pregnanetriol whereas it was usually possible also to detect Δ pregnenetriol which was present in equal or slightly greater amounts than the former In the same specimens, however there was often found Δ -pregnene-3β 16α diol 20 one and this compound was often the predominant one This same compound can be found in the urine of normal newborn infants After the age of 1 month pregnanetriol predominated among the C-21 steroids But as concerns only the triols there was no instance wherein pregnenetriol far outweighed the pregnanetriol And after the first month of life there appeared to be no ambiguity in this group a great predominance of pregnanetriol occurred

Although in younger subjects with 3β hydroxysteroid dehydrogenase deficiency the pregnenetriol clearly predominated with little or no pregnanetriol As the patients became older a more substantial quantity of pregnanetriol appeared This has been noted by others Despite this fact the ratio of pregnenetriol to pregnanetriol was always greater than in the more common type and the two groups could be separated without any overlap There were two other notable differences Pregnanetriolone was commonly found in 21 hydroxylase deficiency but not in 3β ol

tity might best be understood through the hypothesis that all human beings have both a masculine and feminine pattern of gender behavior coded in the brain one of them usually negatively coded the other positively The anomalies of gender identity represent an imbalance of the usual positive negative ratio

H Wallis (Zurich) *Some remarks about practical problems in the psychological management of children with intersexuality*

Although exp rts agree that in treatment of children with intersexuality psychological aspects must always be regarded alongside the physical aspects this is not always or not sufficiently done in practice Many physicians do not know in what way they should talk over these special problems with laymen and especially with children and to what degree they can be frank about the problem

It is therefore intended to illustrate in this discourse some practical aspects of these problems for instance Which time and method should be chosen to explain to the parents the nature of their child s disturbance Method of discussion with the parents about the decisions that are to be made How does one behave towards brothers and sisters relatives and the rest of the community? Which time and method should be chosen for explanation to the child of the nature of his disturbance Kind of treatment necessary and the results and consequences of it Help through illustrated material and texts that can be given to the children Continuous advice when practical difficulties occur

M I New D Dixon K Hirschhorn & B Shannon Danes (New York) *Testosterone metabolism by cultures of human cells*

Testosterone metabolism has been demonstrated in cultures of fibroblasts and amniotic fluid cells Radiolabelled testosterone was incubated with cells in culture for 48 hrs without addition of co factors By a reverse labelling technique the metabolites were identified and purified to constant specific activity by sequential chromatography End time additions of testosterone were used as controls DNA was measured in the cell cultures to control cell number Cultured fibroblasts metabolized testosterone to Δ^4 androstenedione (Δ^4) androsterone (A) dihydrotestosterone (DHT) 3α and 3β androstanediol (Adiol) and androstanedione (Adione) Etiocholanolone was not identified indicating no 5β reduction of testosterone Fibroblast cultures obtained from adult females and males showed a similar pattern of metabolism in which the 17 ketonic pathway was predominantly utilized Fibroblasts from children metabolized testosterone mainly via the 17β hydroxyl pathway but male cells were more active than female cells There was a change in cellular metabolism of testosterone with age i e young cells showed primarily 5α reductase activity while in adult cells the 17β-dehydrogenase activity predominated When the cellular metabolism of children with testicular feminization (TF) was compared to that of age and sex matched normal children there was a great difference The TF fibroblasts produced significantly lower amounts of the 5α reduced metabolites (6 3 %) than did the control (80 %) thus suggesting that there may be decreased enzymatic activity of testosterone 5α reductase in the TF cells Amniotic fluid cells cultured from male fetuses with a gestational age of 15–17 weeks metabolized testosterone similarly to fibroblasts from young children The main metabolites were DHT and Adiol The study of metabolism of hormones in cell culture may provide a tool for the prenatal diagnosis of genetic and endocrine disorders without interruption of pregnancy and for postnatal diagnosis of intersex disorders in which there is an enzymatic deficiency The study of hormone metabolism in cell cultures may also reveal a maturation of target cells with advancing age and puberty

LH), there is an unusual frequency of associated neurological disorders 2) Changes in biosynthesis influenced by circulating androgens or estrogens 3) Modification of the skin s ability to reduce testosterone to androgenic products with the 5 α configuration

Premature thelarche in girls is another puberal variant accompanied by none of the other signs of estrogen action in the vagina or in the skeleton Plasma LH and FSH are probably normal Nevertheless a minimal modification in estrogen secretion from adrenals or ovaries cannot be excluded Gynecomastia developing in the adolescent period is quite common It occurs perhaps in all boys to a minimal degree around the period of growth spurt Its mechanism is unknown

Growth spurt in both sexes has always been observed in careful longitudinal studies It is absent when gonads are dysgenetic or not stimulated In normal puberty it occurs after stage 2 for pubic hair and breasts when gonads and adrenal show characteristic pubescent activity Thus estrogens androgens and probably growth hormone are involved It is likely that testosterone is responsible for the increased growth spurt in boys The role of the adrenal androgens is not known and should be discussed in some cases of premature pubarche there is a slight advance of growth and skeletal maturation More dramatic was the rapid and severe aggravation of paralytic scoliosis simultaneous with the development of premature pubarche in a group we studied One could speculate on selective response of the vertebral cartilage to adrenal androgens Growth hormone is probably necessary and has a permissive effect for the androgenic action as evidenced from studies in delayed puberty and hyposomatrophic dwarfs It is also possible that testosterone and (or) other androgens stimulate growth hormone secretion during normal puberty

In spite of extensive studies our present knowledge of the physiology of the initial stages of puberty is incomplete and deserves critical attention

J Money (Baltimore) *Development of sexual identification*

Intersexuality is theoretically important in demonstrating that the differentiation of gender identity is not preordained by genetic or chromosomal sex directly nor by fetal hormonal sex Fetal androgen, however is an important contributor in that its pressure induces masculinization of the external genitalia and of certain hypothalamic functions both of which would otherwise differentiate as female The external genital appearance profoundly influences the behavior of others from the time of sex assignment throughout rearing as well as the individual s own body image and self conception The hormone differentiated hypothalamus probably influences such sexually dimorphic behavioral differences as energy-expenditure level pelvic thrusting movements in childhood play and dominance assertiveness in the power hierarchy of childhood play In human beings postnatal differentiation of gender identity is not preordained by prenatal hormonal influences though it may be influenced by them Postnatal differentiation like language acquisition, requires social interaction It may issue in a gender identity that is congruent with its antecedents contradictory of them or ambiguous

Postnatal gender identity once differentiated is singularly tenacious in maintaining itself For this reason, the decision concerning an intersexual child s sex assignment should be fixed at the time of birth If later considerations lead to a revision of diagnosis and the possibility of sex reassignment then more attention should be given to the status of the gender identity already differentiated than to the other variables of sex Psychologic failure of sex reassignment in intersexuality is guaranteed unless gender identity has been incomplete or ambiguous in the sex of assignment It is most successful if the intersexual child has already reached a resolution for sex change

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D Gupta E McCafferty & K Rager (Tubingen) *Plasma dihydrotestosterone in adolescent males at different stages of sexual maturation*

For certain tissues such as prostate seminal vesicle, penis and clitoris dihydrotestosterone (5α androstan 17β ol 3 one) a reduction product of testosterone is more firmly bound to nuclear chromatin than is testosterone itself (1)

Dihydrotestosterone (DHT) was estimated by competitive protein binding radio assay in the plasma of adolescent males at different stages of sexual maturation A diethyl ether extract of the alkaline plasma was chromatographed on a precoated silica gel plate followed by chromatography on a 1 5 cm wide methanol washed paper Percentage binding of the steroid was done by interaction with a pooled late pregnancy plasma Charcoal coated dextran was used for separating bound from free Testosterone was measured simultaneously by a similar technique and the validity of the results obtained compared favourably with results from GLC The method interfering factors (MIF) in this assay (corresponding to 300 mm² paper area) gave 0 18 ng of DHT like activity above which level the steroid can be satisfactorily estimated

The 46 children examined were all hospitalized normal children without frank endocrine dysfunction They were graded from stages 1–5 in relation to their sexual maturation The mean 5α DHT concentration in plasma are 3 5 ± 3 2 ng at stages 1 + 2 10 4 ± 4 3 ng at stage 3 25 1 ± 6 8 ng at stage 4 37 8 ± 7 1 ng at stage 5 In 10 adult males the level was 64 7 ± 13 7 ng The difference between the plasma concentrations between individual stages (except stages 1 and 2) and between stage 5 boys and adult males were significant

With administration of HCG to the boys suffering from undescended testes increments in the levels of plasma DHT and testosterone were observed which could explain the significant decrement in the percentage binding of testosterone in the plasma observed earlier (2)

Estimation of DHT may therefore be included as one of the major tests for the assessment of the physiologic status of the gonads in patients suspected of having gonadal dysfunction and treated with HCG

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2 Gupta D Huenges R & Rager K *Acta Endocr* Suppl 152 24 1971

M Zachmann W Hamilton J A Vollmin & A Prader (Zurich and Glasgow) *Testicular 17 20 desmolase deficiency causing male pseudohermaphroditism*

Two male first cousins (XY) presented with ambiguous genitalia A maternal aunt is an XY individual with testicles (removed) rudimentary uterus and one Fallopian tube The infants had no salt wasting Steroid studies were carried out at age 1 8 and 2 4 (subject A) and 2 2 (B) years Both children excreted low quantities of testosterone Urinary pregnanediol pregnanetriol and cortisol metabolites (THF THE) were normal THS and THB detectable No dehydroepiandrosterone was present Pregnanetriolone usually found only in 21 hydroxylase deficiency was present in small amounts 1 3 and 5 days after HCG (5 000 U/m) testosterone remained unchanged and dehydroepiandrosterone undetectable Pregnanetriol also did not change but pregnanetriolone increased More detailed studies were carried out in A the steroid converting capacity of testicular tissue (surgical biopsy) was studied with a double substrate system in separate flasks tissue was incubated with ^{3}H pregnenolone/^{14}C progesterone with ^{3}H 17α hydroxypregnenolone/^{14}C-17α hydroxyprogesterone and with ^{3}H dehydroepiandrosterone/^{14}C androstenedione Androstenedione and dehydroepiandrosterone were converted freely to

testosterone excluding 17 reductase deficiency Testosterone was not formed from any of the other substrates Both pregnenolone/progesterone and the respective 17α hydroxylated compounds the former less than the latter yielded compound S and its 20α and 20β reduced products

Our findings suggest a defect of the side chain cleavage in both the delta-4 and delta 5 pathways Such a 17 20-desmolase defect has not so far been described A case of partial deficiency (delta-4 pathway) associated with 3β hydroxysteroid dehydrogenase deficiency has been reported (1) In our case the low pregnenetriol excretion excludes additional 3β hydroxysteroid dehydrogenase deficiency The occurrence of urinary pregnanetriolone in the presence of a normal 21 hydroxylation and the detection of 17α 20α 21 trihydroxypregn-4-en 3-one (from S) after incubation suggest a secondarily increased activity of the 20α hydroxysteroid dehydrogenase

Reference

1 De Peretti, E & Loras B *Acta Paediat Scand* 58 667 1969

M Ritzen & F S French (Stockholm and Chapel Hill) *Deficient nuclear binding of ^3H testosterone to cell nuclei from a male pseudohermaphrodite rat*

The Stanley Grumbach male pseudohermaphrodite rat mimicks the human syndrome of testicular feminization in several respects in spite of considerable blood levels of testosterone 50 of the male offspring of a seemingly normal heterozygous female fail to virilize *in utero* and typical target organs show no response to large doses of administered testosterone The present studies were undertaken to investigate whether the decreased response to androgenic hormones was due to deficient binding of the hormone to certain cell components Following s c injection of testosterone 1 2 ^3H radioactivity was extracted from several tissues of castrated male pseudohermaphrodite (PS) and control male rats No difference in concentration of radioactivity was found at 30 and 60 min after injection while at 4 hr the retention was higher in the PS organs The kidneys were homogenized and fractionated by differential centrifugation the radioactivity was extracted and isolated by thin layer chromatography No qualitative or quantitative difference in cytoplasmic androgen binding proteins was found when these were studied by sucrose density gradient centrifugation However the purified PS nuclei contained only about 5 % of the testosterone of the control nuclei demonstrating a marked deficiency in nuclear uptake or retention of this hormone The metabolism of administered testosterone 1 2 ^3H was found to be altered in the PS rat kidneys in that there seemed to be a more rapid reduction to androsterone and 5α androstane 3α 17β diol We suggest that this decreased nuclear binding is of importance for the end organ insensitivity to androgenic hormones characteristic of these rats which resemble testicular feminization

L David J M Saez & R François (Lyon) *Male pseudohermaphroditism in two brothers*

This report illustrates the difficulties of classifying some of the observations on male pseudohermaphroditism and demonstrates that it is always necessary to note all the features before establishing a definite diagnosis of the exact type of male pseudohermaphroditism

The following two cases have previously been reported (1) variously as mixed gonadal dysgenesis (MGD) and internal male pseudohermaphroditism (IMPH) when only gonadal histology was considered

The eldest brother is a 10 year-old boy with bilateral cryptorchidism and normal external genitalia The karyotype is 46/XY At laparotomy a uterus bilateral Fallopian tubes and two deferens were found The two gonads could not be lowered and were removed On histological examination the right gonad was a normal testis but the left gonad consisted

of fibrous tissue containing some seminiferous tubules with a Sertoli s cells syncytium The diagnosis of MGD was established by the histo logists on these findings

The younger brother has two testes with unilateral cryptorchidism, inguinal hernia of the uterus and coexistence of male and female internal genital ducts The karyotype is 46/XY The cryptorchid testis was lowered and a hysterectomy was performed with removal of the two deferens An HCG test (2) showed that the testes functioned well as considered by the plasma levels of dehydroepiandrosterone epi androsterone androstenedione and testoste rone

The following comments are relevant

1 Normal external genitalia are never pres ent in MGD Wolffian ducts are never present in the side of the streak in MGD and the pres ence of a deferens proves that the left gonad was not a streak but a testis otherwise the his tological findings are consistent with a very dysgenetic testis as is observed in some cases of IMPH

2 The familial association of two IMPH is in favour of a genetic absence of the inhibiting substance or of a genetic insensibility of the Mullerian ducts to this substance

3 It is a recognized practice to perform a hysterectomy in these patients who neverthe less are sometimes fertile Unfortunately as in our second observation the hysterectomy removed the deferens which were partly en closed in the uterus It might therefore be better to preserve the uterus in these subjects and thus also their possible fertility

References

1 Salle B & Hedinger C *Acta Endocr 64* 211 1970
2 Saz, J M & Bertrand J *Steroids 12* 749 1968

W Hamilton (Glasgow) *The nature of the enzyme defect in salt losing and non salt losing forms of C21 hydroxylase deficiency*

Until the present the salt losing and the non salt losing forms of 21-hydroxylase defect have been regarded as representing a complete and a partial defect respectively of the enzym On biochemical considerations it seems more reasonable that in the nonsalt losing form 21 hydroxylation of 17α hydroxyprogesterone is defective while in the salt losing form 21 hydroxylation of both progresterone and 17α hydroxyprogesterone is defective

To test this hypothesis the following test was devised Three salt losers and three non salt losers were given I M corticotrophin 60 mg on the first test day Also on the first test day and on the day following all subjects re ceived metyrapone 500–750 mg four hourly for twelve doses Urine was collected on the day preceding the test and on the two test days Glucocorticosteroids were withdrawn during the test The urine samples were analysed for pregnanediol and pregnanetriol according to the method of Goldzieher & Nakamura (1)

Rationale

By blocking 11β hydroxylase in a system al ready blocked genetically at C 21 the only metabolites likely to be obtained in quantity would be pregnanediol and pregnanetriol. Since the patients were already suppressed by exogenous steroids it was necessary to give an adrenal stimulus to ensure secretory ac tivity hence corticotrophin was used

It might have been expected in this experi mental situation that nonsalt losers would ex crete large quantities of pregnanetriol while salt losers would excrete large quantities of both pregnanediol and pregnanetriol

Results

Sex	Age (yrs)	Micrograms per 24 hours Pregnanediol	Pregnanetriol
Salt losers			
F	3	700	995
F	4	242	499
F	14	184	638
Non salt losers			
F	2	1 769	3 096
F	6	2 506	3 396
F	8	3 164	10 532

	Days of Na diet										
	1–5 (65 mEq)	6–17 (10 mEq)		18–28 (10 mEq) dexa		29–30 (10 mEq) dexa ACTH		31–32 (10 mEq) dexa.		Surgery	36
Jac 6 years											
Plasma											
11-desoxyCS (μg/100 ml)	15 5	17 6	14 9	13 1	2 5		11 7				12 4
Renin (ng/ml/min)	2		2	0	20	26		74			21
Urine											
Aldosterone (μg/day)	0 7	0 4	0 2	0 5	3 3		16 2	8 6	7 1		2 6
17 KGS (mg/day)	22 0	19 8	13	5 2	0 9	2 9	5 2	5 4			
Jos 4 years											
11-desoxyCS	9 25	4 25	9 1	9 6	5 1		11 5				4 5
Renin	0		0	8	68	33		72			20
Aldosterone	0 1	0 2	0 6	0 3	24 9		24 6	10 6	8 7		5 0
17 KGS	11 1	7 8	11 8	3 4	2 6	2 9	4 7	6 0	7 9		

Clearly in both salt losers and nonsalt losers 21 hydroxylation of progesterone and 17α hydroxyprogesterone is defective and probably equally so What seems to emerge is that in the nonsalt losers there is a greater reserve of substrate and hence if a fixed proportion of substrate is converted to the definite hormones a greater quantity of both aldosterone and cortisol is synthesized Crises are therefore absent In salt losers the quantity of aldosterone and cortisol synthesized is essentially inadequate and therefore adrenal crises follow

It is thus concluded that 21 hydroxylase only is defective (not absent) in salt losers and nonsalt losers for both progesterone and 17α hydroxyprogesterone The essential difference is in the availability of these two substrates in the respective types

Reference

1 Goldzieher J W & Nakamura Y *Acta Endocr* 41 371 1962

P C Sizonenko A M Riondel I J Kohlberg & L Paunier (Geneva) *Adrenal corticosteroids secretions and excretions in relation to electrolyte balance in 11 β hydroxylase deficiency*

Two female pseudo hermaphrodite siblings aged 6 and 4 years raised as brothers and presented with typical adrenal 11 β hydroxylase deficiency They were investigated during high and low sodium intake before and after initiation of therapy

During low sodium diet sodium balance was achieved However aldosterone excretion and plasma renin activity (PRA) were undetectable With dexamethasone therapy and low sodium diet the balance was negative during the first week Thereafter PRA and aldosterone excretion rose and sodium balance was slightly positive The juxta glomerular apparatus was hypoplastic at surgery Plasma cortisol levels were persistently low plasma 11-desoxy corticosteroid levels were high before treatment decreased with therapy and rose following ACTH administration Aldosterone and desoxycorticosterone secretions increased during the combined administration of low sodium diet dexamethasone and ACTH

R P Zurbrugg O H Oetliker A Chattas & E Gugler (Aarau) *Renal tubular acidosis in salt losing syndrome (SLS) of congenital adrenal hyperplasia (CAH)*

A 22 month old patient was studied both in a decompensated state (plasma Na^+ 123 mEq/l plasma HCO_3^- 14 mmoles/l) five days after withholding 9 alpha fluorohydrocortisone substitution and in the compensated state (Na^+ 137 HCO_3 21,6) one week after reinstitution of therapy Renal HCO_3^- threshold was 13 mmoles HCO_3^-/l plasma in decompensation and 20 5 in compensation Since HCO_3^- reabsorption is coupled with Na^+ reabsorption this finding documented mineralocorticoid action on both HCO_3^- and Na reabsorption in the proximal tubule in man as well (1)

A second patient 4 years and 10 months of age was investigated who as usually observed with increasing age seemed to be equilibrated with glucocorticoid (GC) substitution alone Since renal HCO_3^- threshold was found in the normal range the hypothesis was advanced that a volume contraction might increase HCO_3^- threshold and thereby counteract renal Na^+ and HCO_3^- loss

In a third patient 12 years of age who again seemed to be independent of mineralocorticoid (MC) substitution the plasma volumes were measured Without MC substitution plasma Na^+ and HCO_3^- were only slightly below normal HCO_3^- threshold was found at 18 5 HCO_3^- mmoles/l plasma representing a mild acidosis only in comparison to the decompensated state in the still MC dependent first patient 22 months of age When MC substitution was reinstituted plasma Na and HCO_3^- concentrations as well as renal HCO_3 threshold (24 mmoles/l) increased to an absolutely normal range Whereas without MC substitution a low plasma volume of 21 ml/kg was found a 67 % increase to a normal plasma volume of 35 ml/kg could be demonstrated during MC treatment Thus as speculated before in the absence of MC substitution a volume contraction leading to relative compensation, could have effectively counteracted a much more severe HCO_3^- and Na^+ loss Volume expansion itself impairs HCO_3^- an Na^+ reabsorption however the regulatory effect of the relative volume expansion accompanying MC treatment is obviously overcome by the much stronger stimulation of the administered MC

Summary

1 Action in the proximal tubule was also demonstrated in man
2 In aldosterone deficiency volume contraction may counteract HCO_3^- and Na loss as a regulatory mechanism
3 In impaired aldosterone synthesis it might be necessary to maintain MC substitution since these patients are constantly in a dangerously unstable equilibrium their volume contraction being already rather critical

Reference

1 Oetliker O H & Zurbrugg R P Renal tubular acidosis in salt losing syndrome of congenital adrenal hyperplasia (CAH) *J Clin Endocr 31* 447 1970

J M Limal R Rosenfeld J Letarte G Leboeuf & J R Ducharme (Montreal) *The effects of oral medroxyprogesterone acetate (MPA) in idiopathic precocious puberty*

Idiopathic precocious puberty produces in addition to early occurrence of secondary sexual characteristics and true menses in girls the risk of premature closure of epiphyses and permanent short stature Treatment of this condition is generally with intramuscular medroxyprogesterone which sometimes causes side effects especially that of accelerated bone maturation To our knowledge there has been no report on the prolonged action of this drug given perorally

We had the opportunity to study 14 cases of idiopathic precocious puberty (13 girls 1 boy) treated with oral MPA at dosage 10 mg 8 hourly and to observe the effects for a period 2 to 10 years Patients were examined every 3 to 6 months for the effects upon stature sexual maturation and bone maturation as well as for urinary levels of estriol and gonadotrophins Plasma FSH and LH were measured in some cases by radioimmunoassay

Oral MPA slowed the progressive advance of secondary sexual characteristics in all cases and stopped menses in girls Urinary estriol was maintained at prepubescent levels (< 10 µg/24 h) and urinary gonadotrophins were suppressed Before treatment skeletal maturation was usually already advanced with respect to her ht age At the onset of therapy accentuation of this discrepancy occurred and persisted during the first months of treatment Subsequently bone and height age generally showed parallel development and in 3 cases bone advance was even reduced with prolonged therapy No undesirable side effects were observed except weight gain

However in a case of polyostotic fibrous dysplasia (McCune Albright syndrome) bone maturation was accelerated under treatment

In conclusion this study suggests 1) that in idiopathic precocious puberty oral MPA is effective in slowing development of secondary sexual characteristics 2) that it has no side effect in long term therapy especially on bone maturation 3) that it may be useful in reducing the advance in bone maturation and occasionally actually improves the prognosis of final stature

B T Rudd P H W Rayner & C G Theodoridis (Birmingham) *Testosterone and estrogen levels of patients with precocious puberty origin and relation to bone age*

Six of ten children (two boys eight girls age range 2 1/2 -7 years) with precocious puberty were stimulated with ACTH and their plasma and urinary steroid patterns studied in relation to their bone age Irrespective of chronological age a significant increase (3-5 fold) in 17-oxosteroids and pregnanetriol was demonstrated after ACTH In all but two of the patients studied this increase in steroid metabolite excretion was not accompanied by increases in either urinary or plasma testosterone

No clear correlation could be demonstrated between advancement of bone age in untreated girls and their plasma or urinary testosterone levels although in some patients the levels of plasma testosterone were elevated for the chronological age (range 0-203 ng/100 ml plasma) In contrast there was a relationship between increasing excretion rates of urinary estrogens (basal conditions) in untreated girls and advancing bone age The elder of the two boys studied on the other hand showed a marked advancement of bone age which coincided with elevated urinary and plasma testosterone levels

It was concluded that patients with precocious puberty also have an early *adrenarche* and that the testosterone produced arises from peripheral conversion of adrenal precursors especially in girls

O Butenandt (Munchen) *Erythrocytic enzyme activities in hypothyroid children*

A low activity of erythrocytic glucose-6 phosphate dehydrogenase was found by several investigators (1 3) in patients with hypothyroidism whereas others (2) did not confirm this Other enzymes have not been studied Therefore the activity of glucose-6-phosphatedehydrogenase (G-6 PDH) glutathionereductase (GR) glutathioneperoxydase (GSH P) lactic dehydrogenase (LDH) pyruvatekinase (PK) and hexokinase (HK) was studied in 12 patients with hypothyroidism before and during treatment

For the determination of the enzyme activity we used the enzyme test combinations prepared by Biochemica/Boehringer (Mannheim) (G-6 PDH LDH PK) or used methods for enzyme assays with NADH (NADPH) as cofactor according to the literature (GR HK GSH P)

The G-6 PDH was subnormal in 5 untreated hypothyroid children but rose to normal or even to supranormal values during treatment The other enzymes tested were lowered in only 3 of these patients but were normalized during treatment

Simultaneously the reticulocytes increased and anemia disappeared

Whereas Root and his co workers (3) thought the activity of the G-6 PDH to be directly related to the metabolic status of the hypothyroid patients we believe that the low enzyme activities represent an aged population of red cells and that the increase of erythrocytic enzyme activities demonstrates an increased function of the bone marrow, i e output of young red cells Thus enzyme changes are secondary to the erythropoiesis which is increased by thyroid hormone (4)

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4 Shirakura T Azuma M & Maekawa T *Blut 21* 240 1970

B D Hermosa & E H Sobel (New York) *Thyroid in the treatment of short stature*

Observations were made on 10 short children aged 4 to 15 years (3 girls and 7 boys) All had retarded bone age and were free of demonstrable organic disease Protein bound iodine or thyroxine iodine was low in 1 and normal in 9 T_3 resin uptake was normal in the 6 tested Thyroidal uptake of ^{131}I (RaI) was low in 1 of 7 tested according to the present standards That low RaI might be ascribable to a preceding metyrapone test All 3 tests were normal in 2 of 5 All were clinically euthyroid but they were treated with 90 to 120 mg desiccated thyroid daily on the premise that response to treatment is the best criterion of thyroid status None developed hyperthyroidism

Growth rates were compared with the mean expected for each child s bone age The rate improved in 8 patients (Student t $p < 0\ 0005$) The 6 children whose control growth rates were below the mean expected rate approached or exceeded the mean on treatment Two of the 4 patients with normal growth rates also grew more rapidly during treatment In each instance the growth response exceeded the amount that could be attributed to seasonal variation

Although a placebo effect has not been excluded the results are interpreted as indicating that the laboratory procedures currently accepted for the evaluation of thyroid function in children do not detect all those who might benefit from thyroid treatment

H Andersen (Copenhagen) *Dense bones in congenital hypothyroidism*

A limited number of osteopetrosis in severely hypothyroid infants have been reported Various etiological factors renal disorders hypervitaminosis D disturbances in parathyroid and calcitoninsecretion etc have been considered

Few if any such cases have been reported in newborn In order to point out possible prenatal factors involved an athyreotic child with normal serum calcium phosphorus and alkaline phosphatase and in whom dense bones were demonstrated immediately after birth was presented

O Westphal & L Wide (Uppsala) *Thyroid stimulating hormone (TSH) in newborn infants*

Methods

A radioimmunoassay using Sephadex coupled antibodies (Immunosorbend technique) was used Blood samples were obtained from umbilical cord and by puncture of the femoral vein Forty eight infants have been studied during the first week of life Four cases of congenital hypothyroidism have been followed during the first 2 weeks of treatment with 0 025 mg Levothyroxin

Results

Tables 1 and 2 summarize the results

Table 1

Age	Delivery	n	TSH mU/ml Mean	Range
0–24 hours	Spontaneous	10	50 5	20–86
0–24 hours	Caes sec asphyx	4	111.2	87–150
0–24 hours	Caes sec nonasph	4	52	26–98
24–48 hours	Spontaneous	10	32 1	15–59
3 days	Spontaneous	10	25 8	15–32
5–6 days	Spontaneous	10	23 5	15–32
Normal adults				8–40

Table 2

Age at diagnosis	Range of TSH levels mU/ml before treatment	day 3	day 14
2 12 weeks	200– > 220	22–82	4–8

J L Chaussain E Binet & J C Job (Paris) *Anti HTSH antibody like activity in plasma of certain hypopituitary patients*

Radioimmunoassay of TSH in plasma of 40 hypopituitary dwarfs showed in 3 of them a paradoxical decrease of the bound/free radio activity ratio These three patients had been operated for craniopharyngioma As all three had evidence of panhypopituitarism and re ceived thyroid replacement therapy it was speculated that the observed decrease could be associated with anti TSH activity of their plasma although only two of them had pre viously received injections of exogenous thyro tropin

Antibody like activity in these three plasmas was demonstrated by incubation for 5 days with [1] I labelled HTSH Bound and free radio-activity in the incubates was separated in the following two ways

1) chromatoelectrophoresis using Whatman D E 81 paper strips (efficiency of this separa tion method has been experienced by previous works

2) selective precipitation of the bound frac tion by anti human globulin horse serum which also shows the localization of the anti body like activity in plasma globulins

The capacity of these three plasmas to bind [1] I HTSH is quantitatively inhibited by un labelled HTSH but titration of the antibody like activity has not yet been possible Control plasmas do not show ability to bind [1] I HTSH

The exact significance of these facts was discussed and is actually the subject of further investigation

J C Job & E Binet (Paris) *Increase of plasma TSH after intravenous injection of TRH (thyrotropin releasing hormone) in chil dren normal hypothyroid hypopituitary and goitrous*

Results of the TSH stimulation test with intra venous TRH were evaluated in children aged 2 to 17 years A single 200 μg dose of syn thetic TRH was intravenously injected after an overnight fast Venous blood was collected from 0 to 120 min after injection Plasma TSH was radioimmunoassayed with a double anti body method

In normal children quick injection and slow infusion of TRH have been compared Mean values obtained after TRH quick injection at time 0 in 16 children (A) and after slow in fusion from time 0 to time 60 min in 9 chil dren (B) were minutes

	0	10	20	30	40	60	90	120	180
TSH (A)	5 4	17 1	18 9	17 9	15 2	12 5	9 4	7 1	
(B)	5 5		8 3	9 3		14 7	13 2	11 2	7 8

These facts suggest that TRH test offers a valuable assessment of TSH *reserve in children* and that TSH assay 20 to 30 min after a quick *injection* of *TRH* may be a sufficient evaluation

Other children have been studied after a *quick injection* of TRH at time 0 In 4 hypo*thyroid* children the increment of TSH after TRH was very *high* suggesting a strong increase of TSH *reserve in these* patients whose TSH secretion is permanently elevated In 6 hypopituitary dwarfs TSH reserve was found normal in 4 (2 idiopathic and 2 with unoperated craniopharyngioma) and decreased in 2 In 4 children with apparently euthyroid goiter, *TSH* reserve was mildly elevated in 2 and low in 2

S Leisti & J Perheentupa (Helsinki) *Precision of the insulin tolerance test in children*

The reproducibility of endocrine test responses has not been adequately determined Nevertheless, conclusions have been drawn with the presumption that this precision is high

To establish the precision of the standard insulin test responses this test was performed twice in 80 children examined for abnormal growth The responses in concentration of blood glucose plasma cortisol and somatotropin, and in urinary epinephrine excretion rate were determined using a glucose oxidase method fluorimetry radioimmunoassay with carbon dextran separation and the method of Crout, respectively After an overnight fast an indwelling catheter was placed in a cubital vein 30 to 60 min before starting urine and blood collection Regular insulin was given 4 U per m Hypoglycaemic symptoms were observed in every test The samples from the two tests were assayed together, as a rule

The basal levels of cortisol and epinephrine were significantly higher at the first test than at the second The precision of the (hypoglycaemic) glucose response was lower than that of basal glucose concentration (21%) The precision was similar whether the response was measured as a fraction of the basal glucose concentration (27%) absolute nadir (32%) or absolute decrement (30%) For evaluation of the endocrine responses the series was divided in groups I–III in order of decreasing precision of glucose response The precision of glucose recovery index (20%) was similar to that of basal glucose concentration and the same whether the index was measured as a percentage of the basal concentration or in mg/100 ml The precision of basal cortisol concentration (31%) was markedly lower than that of glucose The precision of cortisol response was highest (23%) and similar to that of basal glucose concentration when evaluated as highest absolute cortisol concentration obtained The precision of somatotropin response was poor as assessed on the arithmetic scale (114%) but markedly higher on the log scale (43%) The same was true for the epinephrine response The precision of this was markedly higher when measured on absolute rate of excretion during the test (47% on linear 28% on log scale) than when measured on relative increment as has been recommended earlier

Comparing the groups I–III, the precision of the recovery index and of the responses in cortisol concentration and epinephrine excretion decreased slightly in parallel to the precision of the glucose response However, the intraindividual differences in recovery index and in cortisol and epinephrine responses were discordant as often as they were concordant with the corresponding differences in glucose response

In 14 test pairs a larger than 50% decrease from the fasting blood glucose level was obtained in one but not in the other test Even in this series the difference in somatotropin response was discordant with that of hypoglycaemia in 7 test pairs

The following conclusions were offered 1) For evaluation of the response to hypoglycae-

mia in plasma cortisol and somatotropin and in epinephrine excretion maximum levels of these are to be preferred as their precision is highest 2) The diagnosis of endocrine deficiency cannot be based on one subnormal insulin test response This is particularly true for somatotropin 3) There is no ground to setting a limit to acceptable hypoglycaemia such as 50 fall from the fasting blood glucose level

K A Zuppinger J P Jacot D Donath & E E Joss (Bern) *Evidence of a decreased threshold for insulin secretion in juvenile obesity*

Threshold studies of insulin secretion were performed in 12 obese children (age 4–13 years) with a relative weight of 161–222 ′ Intravenous glucose was administered over a 1 min period at increasing doses (0 02 0 04 0 08 0 33 g/kg) in hourly intervals Plasma glucose and immunoreactive serum insulin (IRI) were determined at 2 5 10 15 20 30 and 60 min after each dose The control group consisted of 12 lean volunteers (age 13–36 years) with a relative weight of 89–107 and they were matched on an equivalent weight basis The total amount of glucose injected therefore was comparable in each pair of individuals

Plasma glucose was significantly lower in the obese group during the three lower doses but equal during the high doses The K value for glucose disappearance determined after the high dose was identical (K obese = 1 52 ± 0 52 K contrast 1 49 ± 0 48) Serum IRI IRI/glucose ratios and the increase of IRI above the preinjection level (ΔIRI) was significantly greater in the obese group at all doses At the lowest dose $\Delta IRI_{0\ 02}$ was only small in the contrast group (12 4 ± 8 0 μU/ml) but already quite distinct in the obese group (30 6 ± 18 1 μU ml $p < 0\ 0025$)

Body composition expressed as lean body mass/total body weight was assessed in all subjects using a liquid scintillation whole body counter for total body potassium measurement

In 6 obese children with a body composition within three standard deviations of our control group $\Delta IRI_{0\ 0}$ was equally small as in the control (15 8 ± 13 2 μU/ml $p = 0\ 25$) In the other 6 obese children with strikingly abnormal body composition $\Delta IRI_{0\ 0}$ was significantly greater (45 3 ± 4 8 μU/ml $p < 0\ 0005$) This group included two patients with Prader Willi Labhart syndrome and one patient with Lawrence Moon Biedl syndrome This finding suggests that in some obese children with strikingly abnormal body composition the threshold for insulin secretion is indeed diminished

B Weber & R Muller Hess (Berlin) *Plasma insulin response versus clinical course in juvenile diabetics*

During a phase of metabolic stabilisation following the manifestation even of ketotic diabetes many children exhibit a postinitial improvement of glucose tolerance which correlates well with a marked reduction of insulin requirements (1) A few children need not be treated at all with exogenous insulin for a varying length of time

In seven diabetic children to whom insulin could be withheld for 8 weeks up to several months either primarily (6/7) or after initial treatment (1/7) blood glucose and plasma insulin levels (radioimmunoassay) following 4 hr glucose loads alone or together with 5 mg glibenclamide orally were measured repeatedly Four of these children were moderately obese In two of the remaining slim subjects insulin treatment became necessary after a 2 month lag period The others after periods of 5 to 9 months following the manifestation of the disease still remained compensated on diet alone

Only three of these latter five children demonstrated slowly increasing plasma insulin values of little more than 20 μU/ml 2 hr or more after glucose or glucose + glibenclamide administration in spite of blood glucose maxima attaining 400 mg/100 ml Neither glucose

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The basal levels of cortisol and epinephrine were significantly higher at the first test than at the second The precision of the (hypoglycaemic) glucose response was lower than that of basal glucose concentration (21%) The precision was similar whether the response was measured as a fraction of the basal glucose concentration (27%) absolute nadir (32%), or absolute decrement (30%) For evaluation of the endocrine responses the series was divided in groups I–III in order of decreasing precision of glucose response Th precision of glucose recovery index (70%) was similar to that of basal glucose concentration and the same whether the index was measured as a percentage of the basal concentration or in mg/100 ml The precision of basal cortisol concentration (31%) was markedly lower than that of glucose The precision of cortisol response was highest (23%) and similar to that of basal glucose concentration when evaluated as highest absolute cortisol concentration obtained The precision of somatotropin response was poor as assessed on the arithmetic scale (114%) but markedly higher on the log scale (43%) The same was true for the epinephrine response The precision of this was markedly higher when measured on absolute rate of excretion during the test (47% on linear 28% on log scale) than when measured on relative increment as has been recommended earlier

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The following conclusions were offered 1) For evaluation of the response to hypoglycae

investigated GH was measured by radioimmunoassay

Addition of cAMP 4×10^{-3} M to the incubation media augmented GH release from isolated adenohypophyses to 3 59 μg/mg/2 hr±0 62 (S E M) vs 2 16±0 72 μg/mg/2 hr in the controls Theophylline 0.5×10^{-3} M increased GH release even more markedly (4 88±0 34 μg/mg/2 hr vs 1 42±0 15 μg/mg/2 hr) Dexamethasone was without effect on spontaneous and on theophylline stimulated GH release *in vitro*

After *in vivo* treatment with dexamethasone and alpha 1 18 NH_2-ACTH no significant change in pituitary GH concentration was detectable (57 2±3 1 μg/mg vs 57 9±4 4 μg/mg and 65 4+4 3 μg/mg vs 73 6±4 0 μg/mg *respectively*) The *in vitro* GH release however was found reduced significantly After dexamethasone pretreatment the value was 2 12±0 18 μg/mg/2 hr instead of 3 07±0 27 for the untreated animals and after administration of alpha 1 18 NH_2-ACTH 1 72±0 13 vs 2 18±1 15 μg/mg/2 hr

M Stahl J Girard & M Vest (Basel) *Investigation on the immunogenic properties of human chronic gonadotrophin in HCG treated children*

HCG has been widely used for the treatment of undescended testes The usefulness of such a hormone therapy is subject to criticism One of the possible hazards of HCG therapy is the induction of antibodies which could crossreact with endogenous LH and/or FSH and therefore affect the function of the testes In animal experiences an inactivation of the LH and FSH by anti HCG antibodies has been demonstrated Anti HCG antibodies can easily be induced in guinea pigs or rabbits

To our knowledge no immunological investigation after HCG therapy in human has been reported After therapeutic use of other peptide hormones (insulin HGH glucagon ACTH TSH) antibodies which can block the biological activity have been described

In the present study a radioimmunological method was used For iodination a highly purified HCG preparation (13 200 I U /mg) was employed Appropriate controls were set up with serum from untreated children and with rabbit anti HCG antibody respectively Dioxan precipitation was used to separate antibody bound from free hormone

Thirty sera from 27 children between 3 and 12 years of age were investigated The blood samples were taken between several days and several months after the last HCG injection The total HCG doses administered varied between 4 500 and 45 000 I U

Anti HCG antibodies could not be detected in any of the sera tested

In order to explain this surprising result the following points were discussed 1 Methodology (especially dioxan precipitation) 2 Neutralisation of antibodies by compensatory over production of endogenous gonadotrophins 3 Immunotolerance against HCG

F Beas C Castillo & A M Pino (Santiago) *Biological actions of a human placental protein with hormonal properties*

A protein has been isolated from human placenta at term with physicochemical and immunological characteristics and has been defined as different from all known protein hormones

The following biological effects have been observed in experimental animals treated with this protein

1 Increase of weight of the uterus in prepubescent Balb rats with a significant dose–response curve (*r* 09731) This result leads us to suggest the provisional name of utero-trophic placental hormone (UTPH)

2 Local application and systemic injection of UTPH stimulates the growth of pigeon crop sac and a dose–response curve was stabilised using the assay of Riddle & Bate

3 This placental protein inhibits estrogen/progesterone induced mammary development in the ovariectomized rat as evaluated by observations of histological sections dry defatted tissue weighings and nucleic acid analyses

nor insulin values of the diabetics were influenced by the sulfonylurea compound In control children exhibiting insulin peaks near 70 μU/ml at 30 min after glucose alone glibenclamide, presumably due to its slow intestinal absorption, caused a second insulin peak 2 hr after the oral application, without elevating the insulin maxima The blood sugar declined considerably faster subsequent to the glucose + glibenclamide load

Even though fasting and maximal blood sugar values improved in one obese boy during several months of dietary treatment and the glucose tolerance was normal following a marked weight loss no amelioration of the insulin response was observed Glibenclamide at this time induced a faster decline of the glucose curve in three subjects without influencing insulin plasma levels

From these observations it may be presumed that the amelioration of glucose tolerance during the so called remission phase of juvenile diabetes does not seem to depend upon an improvement of pancreatic insulin secretion The reported data may however indicate an increased peripheral insulin sensitivity in these subjects The sulfonylurea effects on glucose values alone on the other hand may be compatible with an extrapancreatic action of the substance

Reference

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M Karp M Brown & Z Laron (Petach Tikvah) *A mathematical model for oral glucose tolerance test (OGTT) interpretation*

The interpretation of the glucose and the insulin curves in OGTT are still a matter of dispute Among the proposed calculations are peak values at specific time intervals summation of concentrations over a time period sum of increments areas under the curve etc We propose a standardized method to analyze the glucose and insulin area of the OGTT by computer analysis using the quadratic function $Y = a_0 + a_1 x + a x$ which expresses itself in the area (A) formula

$$A = \frac{7y_1 + 12y + 15y_3 + 16y_4 + 15y_6 + 16y_5 + 7y_7}{14}$$

where y_1 to y_7 are the absolute or the $\log_e$ values of the glucose or insulin at 0 30 60 90 120 150 180 min The following groups were analysed (1) 50 normal subjects (2) 10 pts with Juvenile Diabetes on insulin (3) 10 pts with Juvenile Diabetes on peroral therapy (4) 10 pts with obesity and normal OGTT (5) 10 pts with obesity and glucose intolerance The results are presented in the table

Group	*Glucose area*		Insulin area		Glucose/insulin ratio	
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3	1694±224*	33 6±0 83*	363±133	23 8±1 77	34 0±9 8*	8 57±0 60*
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Due to the greater advancement of bone age compared to height age in patients with GH deficiency or those with high IR HGH the use of anabolic steroids is not indicated in the e patients Their use is also not indicated in patients with primary bone defects and Turner syndrome as these do not show an ade quate acceleration in linear growth

M C Postel Vinay (Paris) *Sodium balance aldosterone excretion and secretion rates study of colon receptors to aldosterone in a 9 year-old boy known as a case of pseudohypo-ad enocorticism (PHA)*

The present case published as a PHA (*Ann Ped 39* 2612 1963) was treated until the age of 2 He was on a free sodium intake from 2 to 9 years When studied at 9 his height was 117 cm (−2S D) Blood pressure was normal Plasma urea and electrolytes were normal Glucocorticoid function was normal no opsi uria normal cortisol SR and metopirone test

Hyperaldosteronism and salt loss were pres ent period (*a*) less than 3 mEq/kg/day sodium intake ASR was 1 2 mg/day urinary tetra hydroaldosterone (THAldo) was 250 μg/day period (*b*) 1 ss than 0 3 mEq/kg/day the sodium balance was slightly negative ASR was 2 2 mg/day and THAldo rose to 600 μg/day

Renal function was normal creatinine inu lin PAH uric acid clearances ability to acidify and to concentrate urine No protein uria Glucose Tm was decreased with intermit tent glucosuria The kidney biopsy showed nor mal tubes and some fibrosed glomeruli

Partial sensitivity of renal tube to endoge nous aldosterone was found spironolactone (15 mg/kg/day during 8 days at period (*a*) induced weight loss decreased plasma sodium increased THAldo to 580 μg/day 9-α Fluoro-hydrocortisone given during 4 days at the end of period (*b*) induced a decrease of THAldo from 600 to 200 μg/day

In stools, during period (*a*) very low Na con centrations (0 06 mEq/day) and low Na/K ratio were found Further control studies on normal children are in progress These data can be paralleled with the results obtained on the sigmoid biopsy (Dr Alberti Oxford) the nuclear mineralocorticoid receptor seems to bind aldosterone in the same way as in normal adults

1 Binding of aldosterone to the colon nuclear receptor was normal
2 The studies of electrolytes in stools showed a response of the colon to hyperaldoster onism
3 There was still an incomplete response of the kidney to aldosterone This case raises the problem of adaptation and clinical re covery

R. Steendijk & A Boyde (Amsterdam and London) *Scanning electron microscopic ob servations on the bone lesion in hypophos phataemic rickets*

Microradiographic examination of undecal cified sections from bone in hypophosphat aemic rickets reveals a lack of mineral around osteocyte lacunae This lesion is mainly lo-calised at the side of the lacunae directed to-wards the nearest surface e g an Haversian canal The perilacunar lesion is also visible in decalcified sections when special precipitating stains are used (Schmorl Bodian) The matrix appears globular at these sites suggesting that the normal lamellar arrangement of the col lagen fibres has been replaced by lumps of bone matrix The question arises whether the lesion is the result of incomplete mineralisa tion of the matrix or whether the collagen at these sites is abnormal with resulting impair ment of mineralisation

Scanning electron microscopic observations on undecalcified sections of alcohol fixed bone from a 15 year-old girl with hypophosphat aemic rickets showed that the mineral in the perilacunar areas consisted of separate clusters or lumps with a diameter of 1–3 μm Quite unexpectedly it appeared that the unmineral ised matrix could be removed by treatment of the section with trypsin (2% solution pH 8

4 UTPH does not increase the weight curve of *the hypophysectomized rat (genetic dwarf* rat) A positive result was observed with HGH in the same experimental conditions

5 UTPH does not mobilize NEFA in the fasting animal (rats and rabbits) nor *in vitro*

The above results provide convincing evidence that UTPH possesses hormonal properties providing an important contribution in the growth of the uterus during pregnancy and could explain the well known inhibition of lactation during this period

N Josso (Paris) *Effect of the human testis on the rat Mullerian duct in organ culture*

Though the inhibitory effect of the foetal testis on the Mullerian duct was demonstrated by Jost as early as 1947 little is known of the substance responsible for this effect We have tested the Mullerian inhibiting capacity of the human testis *in vitro* using the rat Mullerian duct as end organ in our bioassay

Testicular tissue was obtained from 30 human foetuses aged 7 to 30 weeks after conception, from 12 subjects in the neo natal period and from 9 older patients (5 months to 20 years) The testicular tissue was associated in organ culture with the castrated reproductive tract of 14 half day old foetal rats of both sexes One hundred and twenty one tracts were associated with foetal 69 with neo natal and 68 with post natal testicular tissue Forty one control *reproductive tracts were cultured* alone In addition 18 tracts were cultured separated from foetal testicular tissue by the vitelline membrane of a hen s egg and 22 separated from the foetal testis by a sheet of Visking cellulose

After 3 days in culture, in the control tracts, the Mullerian duct extended from the ostium to the vicinity of the uro genital sinus In the tracts associated with foetal testicular tissue the Mullerian duct was inhibited only its posterior extremity retaining a lumen Inhibitory activity decreased in the perinatal period and was lost thereafter Inhibition of the Mullerian duct proceeded normally when an egg vitelline membrane was inserted between the rat reproductive tract and the human foetal testis but not when the same experiment was done with a sheet of Visking cellulose This finding indicates that the Mullerian inhibiting substance is non dialysable which is in keeping with the hypothesis that it is a foetal protein no longer synthesized in later life

Z Laron M Torem & A Pertzelan (Petach Tikvah) *The effect of anabolic steroids on linear growth and bone maturation in dwarfism due to HGH deficiency and dwarfism with high plasma IR HGH*

The effects of Methandrostenolone (0 03–0 05 mg/kg/d) and of Fluoxymesterone (0 15–0 2 mg/kg/d) were studied in the following groups of subjects Group I isolated HGH deficiency 6 patients Group II, panhypopituitarism 5 pts Group III dwarfism with high IR HGH 9 pts As controls served Group IV, constitutional growth retardation 20 pts (matched by *age and sex to the previous* patients) Group V primary bone disease, and Group VI gonadal dysgenesis together 12 pts The main results are summarized in the table representing the mean differences (±S D) between treatment and control period

Group	HA/CA	BA/CA	HA/CA–BA/CA	Velocity (obs /exp)
I	0 04 ±0 03*	0 077±0 030*	−0 036±0 026*	0 711±0 457*
II	0 019±0 01*	0 031±0 017*	−0 130±0 020	1 024±1 690
III	0 023±0 017*	0 073±0 054*	−0 050±0 056*	0 706±0 378*
IV	0 044±0 048*	0 045±0 018*	0 030±0 044	0 928±0 582*
V	0 013±0 02	0 031±0 026*	−0 006±0 03	0 113±0 652
VI	0 001+0 017	0 047+0 095	−0 046+0 091	— —

* Statistically significant difference

Due to the greater advancement of bone age compared to height age in patients with GH deficiency or those with high IR HGH the use of anabolic steroids is not indicated in these patients Their use is also not indicated in patients with primary bone defects and Turner syndrome as these do not show an adequate acceleration in linear growth

M C Postel Vinay (Paris) *Sodium balance aldosterone excretion and secretion rates study of colon receptors to aldosterone in a 9 year-old boy known as a case of pseudohypoadrenocorticism (PHA)*

The present case published as a PHA (*Ann Ped 39* 2612 1963) was treated until the age of 2 He was on a free sodium intake from 2 to 9 years When studied at 9 his height was 117 cm (~2S D) Blood pressure was normal Plasma urea and electrolytes were normal Glucocorticoid function was normal no opsiuria normal cortisol SR and metopirone test

Hyperaldosteronism and salt loss were present p riod (*a*) less than 3 mEq/kg/day sodium intake ASR was 1 2 mg/day urinary tetrahydroaldosterone (THAldo) was 250 μg/day period (*b*) less than 0 3 mEq/kg/day the sodium balance was slightly negative ASR was 2 2 mg/day and THAldo *rose to 600* μg/day

Renal function was normal creatinine inulin PAH uric acid clearances ability to acidify and to concentrate urine No proteinuria Glucose Tm was decreased with intermittent glucosuria The kidney biopsy showed normal tubes and some fibrosed *glomeruli*

Partial sensitivity of renal tube to endogenous aldosterone was found spironolactone (15 mg/kg/day during 8 days at period (*a*) induced weight loss decreased plasma sodium increased THAldo to 580 μg/day 9 α Fluorohydrocortisone given during 4 days at the end of period (*b*) induced a decrease of THAldo from 600 to 200 μg/day

In stools during period (*a*) very low Na concentrations (0 06 mEq/day) and low Na/K ratio were found Further control studies on normal children are in progress These data can be paralleled with the results obtained on the sigmoid biopsy (Dr Alberti Oxford) the nuclear mineralocorticoid receptor seems to bind aldosterone in the same way as in normal adults

1 Binding of aldosterone to the colon nuclear receptor was normal
2 The studies of electrolytes in stools showed a response of the colon to hyperaldosteronism
3 There was still an incomplete response of the kidney to aldosterone This case raises the problem of adaptation and clinical recovery

R Steendijk & A Boyde (Amsterdam and London) *Scanning electron microscopic observations on the bone lesion in hypophosphataemic rickets*

Microradiographic examination of undecalcified sections from bone in hypophosphataemic rickets reveals a lack of mineral around osteocyte lacunae This lesion is mainly localised at the side of the lacunae directed towards the nearest surface e g an Haversian canal The perilacunar lesion is also visible in decalcified sections when special precipitating stains are used (Schmorl Bodian) The matrix appears globular at these sites suggesting that the normal lamellar arrangement of the collagen fibres has been replaced by lumps of bone matrix The question arises whether the lesion is the result of incomplete mineralisation of the matrix or whether the collagen at these sites is abnormal with resulting impairment of mineralisation

Scanning electron microscopic observations on undecalcified sections of alcohol fixed bone from a 15 year old girl with hypophosphataemic rickets showed that the mineral in the perilacunar areas consisted of separate clusters or lumps with a diameter of 1–3 μm Quite unexpectedly it appeared that the unmineralised matrix could be removed by treatment of the section with trypsin (2% solution pH 8

4 UTPH does not increase the weight curve of the hypophysectomized rat (genetic dwarf rat) A positive result was observed with HGH in the same experimental conditions

5 UTPH does not mobilize NEFA in the fasting animal (rats and rabbits) nor *in vitro*

The above results *provide convincing* evi dence that UTPH possesses hormonal pro perties providing an important contribution in the growth of the uterus during pregnancy and could explain the well known inhibition of lactation during *this period*

N Josso (Paris) *Effect of the human testis on the rat Mullerian duct in organ culture*

Though the inhibitory effect of the foetal tes tis on the Mullerian duct was demonstrated by Jost as early as 1947, little is known of the sub stance responsible for this effect We have tested the Mullerian inhibiting capacity of the human testis *in vitro* using the rat Mullerian duct as end-organ in our bioassay

Testicular tissue was obtained from 30 human foetuses aged 7 to 30 weeks after con ception from 12 subjects in the neo natal pe riod and from 9 older patients (5 months to 20 years) The testicular tissue was associated in organ culture with the castrated reproduc- tive tract of 14 half day old foetal rats of both sexes One hundred and twenty one tracts were associated with foetal 69 with neo natal and 68 with post natal testicular tissue Forty one control reproductive tracts were cultured alone In addition 18 tracts were cultured separated from foetal testicular tissue by the vitelline membrane of a hen s egg and 22 separated from the foetal testis by a sheet of Visking cellulose

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37°C for 1–2 days) Normal collagen is not soluble in trypsin and therefore this finding may point to an abnormal molecular arrangement of the collagen fibres which at present cannot be further defined

H Stolecke (Bochum) *Congenital adrenal hyperplasia about surgical treatment of total virilized forms A documentation of opinions collected by a questionnaire*

In connection with a personal observation, we studied all cases published up to 1969 with reference to the anatomical conditions of vagina and urethra From the papers studied it appears that

(*a*) A narrow communication between the vagina and urethra in the area of pars prostatica appears with great regularity
(*b*) This meatus is proximal to the sphincter externus
(*c*) The distal part of the vagina is often deformed

For reconstructive surgery therefore the following conditions are essential

1 That the external genitalia are converted to feminine appearance
2 That a good separation of the urethral and vaginal structures is obtained
3 That plastic repair of the high placed vagina results in exteriorization
4 That destruction of the sphincter externus is prevented
5 That an anatomically and functionally well established situation exists after surgical treatment

Last year we issued a questionnaire to thirty two specialists Fourteen questions concerned surgical problems and experience We received twenty two answers Although there was a clear tendency for most authors to favour surgical reversal and to perform it within the first two years of life only three favoured total correction (which implies plastic repair of the vagina at this time

The key for discussion must be seen in the surgical possibilities A satisfying result after surgical treatment can be seen only in a complete feminization according to the above mentioned points Hendren with his team in Boston has developed a surgical method performing all procedures necessary in the first year in one session The patients treated in this manner seem to have a good prognosis. Problems of genito-urinary tract infections are few and the child s awareness of the psychologically burdened surgery is avoided Of course a specialized center is needed to attain full success

J J Heinrich J Colombo & G Bergada (Buenos Aires) *Some observations during treatment of the salt losing form (SLF) of congenital adrenal hyperplasia (CAH)*

Twelve patients (9 females 3 males) were followed up from the neonatal period to ages varying between 4 months and 9 5/12 years They were treated with oral prednisone or hydrocortisone and 9α fluorohydrocortisone (9α f F), or DOCA and salt The following observations were made 1) ECG was carried out in 9 patients before steroid therapy Three of them did not show electrical changes of hyperkalemia even though their serum K^+ levels were between 6 5 and 9 mEq/l while 3 other patients with serum K^+ values between 6 6 and 11 5 showed very mild signs By contrast the other 3 patients who received a large Na^+ intake before admission had narrow and high T waves 2) Growth and bone age were below normal in 11 patients under treatment with prednisone every 12 h or hydrocortisone every 8 h All attempts to decrease the dose were followed by an acceleration of bone age or abnormally high urinary 17 KS or pregnanetriol 3) Eleven of the 12 patients developed hypertension and cardiac signs of hypervolemia of different degrees In all patients the blood pressure became normal after reducing salt intake 4) A 9 year old girl discontinued 9-α f F at age 5 She can maintain normal electrolyte

and water balance even though her aldosterone secretion rate is very low It is concluded that 1) ECG is usually not useful for the diagnosis of hyperkalemia in these patients 2) It is dif ficult to control ACTH secretion without af fecting growth and 3) salt requirements seem to diminish with age

M A Rivarola & E Podesta (Buenos Aires) *Metabolism of ^{14}C testosterone (T) by rat tes ticular tissue*

Testicular tissue from 8 mature Wistar rats was incubated with ^{14}C testosterone for 3 hours at 37 C under 95 % O 5% CO with out addition of cofactors The tissue was pre pared as follows 1) slices of a whole testis 2) tubules of half a testis in duplicate 3) inter stitial cells of half a testis 4) control of ^{14}C T 5) interstitial cells of half a testis incubated with ^{14}C androstendione (Δ) 6) control of ^{14}C Δ After incubation ^{3}H T ^{3}H dihydrotestoster one (DHT) and ^{3}H 5α androstan 3α 17β-diol (DIOL) were added for recovery The tissues were homogenized the steroids extracted with chloroform methanol chromatographed on paper and on a thin layer of silica gel DIOL and T were acetylated before TLC Mean % radioactivity was as follows 1) whole testis T 62 8 DHT 4 8 DIOL 10 1 2) tubules of half testis T 61 0 DHT 3 6 DIOL 11 4 3) inter stitial cells of half testis T 67 2 DHT 2 9 DIOL 1 0 4) control without tissue T 84 5 DHT 0 5 DIOL 0 1 The activity of the inter stitial cells was shown by a 63 3 % conversion of Δ into T while the control converted 0 6 In summary DIOL has been the main iden tified metabolite of T in testicular tissue of rats This conversion takes place in the tubules and very little in the interstitial cells

K E Petersen (Copenhagen) *The value of secretion rate determination in children with adrenocortical diseases*

During the last year doubt has been raised on the assumptions involved in determination of secretion rate for cortisol One group espe cially (Fukushima Hellman Gallagher) has published results showing that the assumption of identity between the specific activity of two or several metabolites of cortisol is not correct in certain patients The concept of a continu ous secretion of cortisol underlying the secre tion rate measurement in this way is also dis cussed

In order to discuss these problems further some results are presented In children with adrenocortical diseases and in other patients we have studied the secretion of cortisol and corticosterone The specific activity was meas ured partly by double isotope derivative tech nique and partly by use of a blue tetrazolium reaction In terms of secretion rate the cortisol value measured from THF most often was lower than the THE value The variations was 10–25% of the THE value in the normal range In the very low values the percentage variation was great and in the higher values (after stimulation with ACTH) it was over 25% When the specific activity of allo-THF has been used the secretion rate in some pa tients was 100 % or more above that from the two other metabolites Corticosterone is often lower than 5 mg/24 h and the difference be tween THB and THA determinations is up to 50 % of the THB value

A Dazord J M Saez & J Bertrand (Lyon) *Problems related to the determination of blood production (BP) of cortisol (F) and cortisone (E) and their interconversion by means of con stant infusion*

A constant infusion of both ^{3}H F and ^{14}C E was given to ten normal subjects (5 males and 5 females)

No significant sex difference was found in the MCR of E and F and their reciprocal in terconversion

But the MCR of E (492 ± 147 l/m /24 h) was significantly higher ($p < 10^{-6}$) than the MCR of F (176 ± 76 l/m /24 h)

The transfer constant $((\rho)_{BB} \times 100)$ for con

version of F to E (59% ± 5 05) was also significantly lower ($p < 10^{-5}$) than that for conversion of E to F (84% ± 8 3)

When comparing the BB of E to the calculated amount of E coming from F (E←F) three situations were observed first BPE was higher than E←F, therefore E was secreted in 25% of the cases second BPE was equal to E←F (35% of the cases) third E←F was higher than BPE (40% of the cases) In these latter cases the blood production of F was the highest and this paradoxical situation might be related to the non steady state in F secretion This was confirmed by the repeated measures of non isotopic F concentrations in plasma during the constant infusion So in three quarters of our cases E was not secreted

In three patients with Cushing syndrome we found a significant increase of both MCRs of E and W while the interconversion F E was normal

In three patients with hyperthyroidism we found a significant increase of the MCRs of F and E and a significant decrease of the transfer constant for conversion of E to F while the conversion of F to E was normal

W Blunck & R P Willig (Hamburg Eppendorf) *Estimation of cortisol in plasma by CPB technique A critical evaluation*

A method for the estimation of plasma cortisol by competitive protein binding (CPB) was established for the study of dynamics of adrenal function in the newborn

10 20 and 40 μl of plasma (capillary blood) are extracted with methylene chloride and then chromatographed on washed silica gel plates in water saturated solvent mixtures (chloroform + 5% ethanol chloroform + 10% ethanol) The transcortin-donator plasma is highly diluted (1 500 1 1 000) free cortisol is removed by 80 mg Florisil

Criteria of reliability according to our data are

1 Precision 5% (for 1 5–10 ng) 8% (for 1 ng) if the estimation is performed on the same day with the same transcortin preparation

2 Accuracy following chromatography is good if the standards are added to the plasma of a patient with Addison s disease and if they are handled exactly like the unknown samples

3 Sensitivity is still limited by unknown factors a good and constant precision is only achieved above 1 ng of cortisol

4 The most important criterion of reliability is a linear correlation of the results using 2 or 3 different amounts of plasma If there is no correlation (range more than 10%) the estimation has to be repeated In our hands this criterion is fulfilled in 5 out of 6 estimations

5 Values less than 5 μg/100 ml are more reliable when chromatography is used since nonspecific chromogens disturb the Porter Silber reaction for the comparable range Above 5 μg/100 ml the correlation between the two methods is good

6 When chromatography is applied 8 double estimations and a standard curve can be performed within 2 ½ days A critical evaluation of the method and a comparison with a similar method without chromatography will be presented Both these procedures will be further compared to other routine methods for cortisol estimation

J M Saez A M Morera & J Bertrand (Lyon) *Testicular endocrine function in children normal and pathological conditions*

In normal children before puberty plasma testosterone (T) levels were very low (31 ± 10 ng/100 ml) but there was a marked increase (554 ± 121 ng/100 ml) following HCG administration (1 500 I U every 2 days for 14 days) The same HCG stimulation has been used in several pathological conditions We regard as a pathological response to HCG a testosterone concentration below the 2nd S D of the mean value obtained in the control group

Over 25 children with male pseudoherma

phroditism 13 have a normal testicular response in the 12 others an insufficient increase of plasma T under HCG was found Among this second group in 2 cases we could demonstrate a testicular defect in 17β hydroxysteroid dehydrogenase

Twenty other children clinically diagnosed as having primary testicular insufficiency were also studied the T response to HCG was normal in 9 of them abnormal in 11

Subjects with Klinefelter syndrome studied before or during puberty showed a normal increase of plasma T after HCG stimulation as well as 2 cases of male Turner syndrome

The plasma concentration of T was also measured in 6 boys with precocious puberty under basal conditions and after testicular suppression Three subjects showed a normal suppression (plasma T decreasing to normal values for children) while the 3 other subjects did not These last 3 cases each had a brain tumour

J Perheentupa A Dessypris & H Adlercreutz (Helsinki) *Plasma testosterone levels in boys following gonadotropin stimulation*

Plasma testosterone concentration (T) was determined in 48 prepubertal boys 0 3–15 7 years old before and after stimulation of the testes with human chorionic gonadotropin (HCG) This was given in three i m injections of 1 500 or 500 I U at 2 day intervals blood being drawn on the day following the last dose T was determined by a competitive protein binding method

Twenty four of the boys were seen because of incomplete descent of testes or delayed maturation and were found to have otherwise normal genital anatomy and at least one testis normal to palpation and bone age (BA) less than 11 5 years Their basal T ranged from nondetectable (less than 15 ng/100 ml) to 65 ng/100 ml with no significant correlation to BA 14 boys of this normal series were stimulated with 3 × 1 500 I U HCG Their T rose to the range of 290–1 100 ng/100 ml mean 520 ng/100 ml and ±2 S D interval 110–930 ng/100 ml The dispersion of the poststimulation levels was logarithmic normal rather than normal On a log scale the mean was 491 ng/100 ml and the ±2 S D interval 254–951 ng/100 ml 11 boys were stimulated with 3 × 500 I U HCG Their stimulated T range was 230–700 ng/100 ml log scale mean 380 ng/100 ml and ±2 S D interval 142–1 015 ng/100 ml This response was lower than that to 3 × 1 500 I U with $p < 0\ 1$ The responses were not correlated to BA the youngest boy age 0 3 years had T 700 ng/100 ml after 3 × 500 I U

Seven boys of BA 11 5–14 5 years with delayed maturation had basal T ranging from nondetectable to 150 ng/100 ml and all stimulation responses in the range of the younger group Of eight boys with hypospadias but at least one testis normal to palpation six had responses in the normal range Two had T 210 ng/100 ml and 320 ng/100 ml on 3 × 1 500 I U HCG i e probably slightly subnormal responses Of six boys with no palpable testes three had normal responses and in three others T of only 0–53 ng/100 ml after 3 × 1 500 I U HCG Of two boys with the clinical diagnosis of the Prader Willi syndrome one had T 122 ng/100 ml on 3 × 1 500 I U and the other T 200 ng/100 ml on 3 × 500 I U A boy diagnosed as having Noonan s syndrome with one testis palpable had T 43 ng/100 ml on 3 × 500 I U

In conclusion determination of plasma testosterone concentration following three i m injections of 500 I U HCG is a test which not only proves or excludes the presence of hormonally functional testicular tissue in prepubertal boys but probably also makes it possible to distinguish between normal and partially defective glands

J R Bierich D Gupta & S Heller (Tubingen) *Examination by cyproterone of the hypothalamo-hypophyseo-gonadal system in prepubertal boys*

Our early observation of 4-fold increase of the initial testosterone excretion and of detectable

levels of urinary gonadotrophins in a 3 year old boy with precocious puberty due to a diencephalic hamartoma when treated with cyproterone led us to investigate, systematically, the effect of this substance on the excretion of the androgen metabolites and gonadotrophins in boys

Cyproterone (100 mg/day) was given to 17 boys aged 5–14 years for a period of 12 days Urinary 17 oxosteroids 17 hydroxycorticosteroids (Few 1961) the individual 11 deoxy 17-oxosteroids testosterone and epitestosterone (Gupta 1969) and gonadotrophins (mouse uterus test after kaolin precip) were estimated before and after the administration of the drug The preliminary data so far obtained from 8 investigations in the present series demonstrate the following no change in the 17 OH-corticosteroids 2–3 fold increase in the 17 oxosteroids, mean increments for androsterone, aetiocholanolone dehydroepiandrosterone testosterone and epitestosterone of 101 % 63 %, 49 % 72 % and 66 % respectively The percentage increments were not different between 5–8 year old boys and 10–14-year-old boys Urinary gonadotrophins increase in 3 questionable alteration in 2 no change in 3 cases

Conclusion 1) A negative feedback mechanism between the gonads and the hypothalamo hypophyseal system exists also in sexually immature boys 2) It is possible to determine the gonadotrophic function of the adenohypophysis in pre pubescent boys by use of cyproterone

PAPERS READ BY TITLE

C C Forsyth D C L Savage E McCafferty & J Cameron (Dundee) *The excretion of individual 17 oxosteroids and corticosteroids in the urine during childhood and adolescence*

There are few reports of accurate fractionation of adrenal metabolites in the urine of children We have studied the individual 17 oxosteroids and the α ketolic metabolites of cortisol and corticosterone during a 24 hour period in 83 normal children and adolescents and in 10 adults by paper chromatography using Bush systems The normal results which have their own physiological interest provide a basis on which the effect of various disease states on adrenal metabolism may be compared

There is an increase from infancy to adult life in the excretion of the total 17 oxosteroids the 11-deoxy 17 oxosteroids (dehydroepiandrosterone aetiocholanolone androsterone) and the 11 oxy 17 oxosteroids (11β hydroxy aetiocholanolone 11β hydroxyandrosterone 11 oxo aetiocholanolone 11 oxoandrosterone) Dehydroepiandrosterone is detectable by this method as early as 6 years of age There is no correlation of the excretion of the 17-oxosteroids the 11 deoxy 17 oxosteroids or the 11 oxy 17 oxosteroids with height weight or surface area from infancy to adult life

The excretion of the 17 hydroxycorticosteroids and the α ketolic metabolites of cortisol (tetrahydrocortisol allo tetrahydrocortisol tetrahydrocortisone) increases from infancy to adult life and both groups of steroids show a correlation with body weight The percentage excretion of allo tetrahydrocortisol falls in early childhood and rises again at puberty

The corticosterone metabolites (tetrahydro corticosterone allo tetrahydrocorticosterone and tetrahydro 11 dehydrocorticosterone) fall in relation to body weight during the first four years of life but correlate with body weight thereafter Allo tetrahydrocorticosterone is the major corticosterone metabolite and its percentage excretion does not show either a rise or fall throughout childhood

There is a preferential degradation of the 17-oxosteroids to 5α derivatives during puberty demonstrated by the rise in the 5α/5β ratio of androsterone aetiocholanolone and 11 hydroxyandrosterone 11 hydroxyaetiocholanolone The 5α/5β ratio of allo-tetrahydrocortisol tetrahydrocortisol also increases at puberty In our series the 5α/5β ratios rise earlier in girls than in boys

H Gleispach H Berger J Glatzl & H Rossel (Innsbruck) *Pregnanetriolone excretion following ACTH stimulation as a possible way for a biochemical proof of heterozygous carriers of an AGS*

This study was carried out on a family with 6 girls 4 of whom have congenital AGS The 2 other girls and also the parents showed no clinical signs of AGS It is assumed that the parents are heterozygous carriers of the gene responsible for the defect in the 21 hydroxylase In the literature we found studies in heterozygotes concerning plasma 17-α hydroxy progesterone and the excretion of pregnanetriol in the urine but no examinations of the metabolite which is significant for this enzyme defect pregnanetriolone In normal persons we could not find pregnanetriolone with our method which is sensitive at 5 μg/d neither as basal excretion nor following stimulation with ACTH According to the results we think that in compensated heterozygous carriers of 21 hydroxylase defect we will not find pregnanetriolone in the basal excretion Following stimulation with ACTH however a part of the higher amount of 17-α hydroxy progesterone may be hydroxylated directly on 11 C and thus pregnanetriolone will be excreted in the urine In our family we found pregnanetriolone after stimulation with ACTH in the urine of the 2 healthy girls and of the parents The father however excreted pregnanetriolone in the basal urine (60 μg/d) We believe that in this family 4 of the 6 girls are homozygotes whereas the 2 others and the moth r are heterozygotes with a partial 21 hydroxylase deficiency We think therefore that the estimation of pregnanetriolone in the urine following ACTH stimulation may be a method for the biochemical identification of symptomless heterozygous carriers of 21 hydroxylase deficiency

J R Guell Gonzalez A Paramio-Rubial & B Delgado-Morales (Havana) *Male pseudohermaphroditism with internal bisexual genitals A report on two brothers*

The cases of two brothers with well-developed Mullerian ducts (tubes and uterus) together with structures derived from the Wolffian ducts and apparently normal external male genitalia and karyotype 46 XY are reported

Nacreous white testis with thickened albuginea prepubertal seminiferous tubules with sparse spermatogonia and interstitial tissue containing abundant fibroblastic elements were found There were no ovarian structures

We consider this condition as a syndrome of well defined clinical characteristics and with a recessive inheritance Its etiology is postulated as a genetic testicular defect by which there is a deficit in the production and/or release of the substance that provokes the involution of the Mullerian structures

We consider the removal of all internal genital structures together with the gonads to be the ideal treatment

Z Laron & H J Hochman (Petach Tikvah) *Small testes in prepubertal boys with Klinefelter syndrome*

In 8 out of 9 children with proven Klinefelter Syndrome it was found that at all ages the testicular volume was below the mean established for normal children (1) The small volume was already evident prepubertally with the youngest boy 2 8/12 years of age and continued to be so during puberty Penile size was variable with a tendency towards low or below normal for established norms

It is suggested that small testes before pu

levels of urinary gonadotrophins in a 3 year old boy with precocious puberty due to a diencephalic hamartoma when treated with cyproterone, led us to investigate systematically the effect of this substance on the excretion of the androgen metabolites and gonadotrophins in boys

Cyproterone (100 mg/day) was given to 17 boys aged 5–14 years for a period of 12 days Urinary 17 oxosteroids, 17 hydroxycorticosteroids (Few 1961) the individual 11 deoxy 17 oxosteroids testosterone and epitestosterone (Gupta 1969) and gonadotrophins (mouse uterus test after kaolin precip) were estimated before and after the administration of the drug The preliminary data so far obtained from 8 investigations in the present series demonstrate the following no change in the 17 OH corticosteroids 2–3 fold increase in the 17 oxosteroids, mean increments for androsterone, aetiocholanolone dehydroepiandrosterone testosterone and epitestosterone of 101 %, 63 %, 49 % 72 % and 66 % respectively The percentage increments were not different between 5–8 year-old boys and 10–14 year-old boys Urinary gonadotrophins increase in 3 questionable alteration in 2 no change in 3 cases

Conclusion 1) A negative feedback mechanism between the gonads and the hypothalamo hypophyseal system exists also in sexually immature boys 2) It is possible to determine the gonadotrophic function of the adenohypophysis in pre pubescent boys by use of cyproterone

PAPERS READ BY TITLE

C C Forsyth D C L Savage E McCafferty & J Cameron (Dundee) *The excretion of individual 17 oxosteroids and corticosteroids in the urine during childhood and adolescence*

There are few reports of accurate fractionation of adrenal metabolites in the urine of children We have studied the individual 17 oxosteroids and the α ketolic metabolites of cortisol and corticosterone during a 24 hour period in 83 normal children and adolescents and in 10 adults by paper chromatography using Bush systems The normal results which have their own physiological interest provide a basis on which the effect of various disease states on adrenal metabolism may be compared

There is an increase from infancy to adult life in the excretion of the total 17 oxosteroids the 11 deoxy 17 oxosteroids (dehydroepiandrosterone, aetiocholanolone androsterone) and the 11 oxy 17 oxosteroids (11β hydroxy aetiocholanolone 11β hydroxyandrosterone 11 oxo aetiocholanolone 11 oxoandrosterone) Dehydroepiandrosterone is detectable by this method as early as 6 years of age There is no correlation of the excretion of the 17-oxosteroids the 11 deoxy 17-oxosteroids or the 11 oxy 17 oxosteroids with height weight or surface area from infancy to adult life

The excretion of the 17 hydroxycorticosteroids and the α ketolic metabolites of cortisol (tetrahydrocortisol allo tetrahydrocortisol tetrahydrocortisone) increases from infancy to adult life and both groups of steroids show a correlation with body weight The percentage excretion of allo tetrahydrocortisol falls in early childhood and rises again at puberty

The corticosterone metabolites (tetrahydrocorticosterone allo tetrahydrocorticosterone and tetrahydro 11-dehydrocorticosterone) fall in relation to body weight during the first four years of life but correlate with body weight thereafter Allo tetrahydrocorticosterone is the major corticosterone metabolite and its percentage excretion does not show either a rise or fall throughout childhood

There is a preferential degradation of the 17-oxosteroids to 5α derivatives during puberty demonstrated by the rise in the 5α/5β ratio of androsterone aetiocholanolone and 11 hydroxyandrosterone 11 hydroxyaetiocholanolone The 5α/5β ratio of allo-tetrahydrocortisol tetrahydrocortisol also increases at puberty In our series the 5α/5β ratios rise earlier in girls than in boys

H Gleispach H Berger J Glatzl & H Rossel (Innsbruck) *Pregnanetriolone excretion following ACTH stimulation as a possible way for a biochemical proof of heterozygous carriers of an AGS*

This study was carried out on a family with 6 girls 4 of whom have congenital AGS The 2 other girls and also the parents showed no clinical signs of AGS It is assumed that the parents are heterozygous carriers of the gene responsible for the defect in the 21 hydroxylase In the literature we found studies in heterozygotes concerning plasma 17-α hydroxy progesterone and the excretion of pregnanetriol in the urine but no examinations of the metabolite which is significant for this enzyme defect pregnanetriolone In normal persons we could not find pregnanetriolone with our method which is sensitive at 5 μg/d neither as basal excretion nor following stimulation with ACTH According to the results we think that in compensated heterozygous carri rs of 21 hydroxylase defect we will not find pregnanetriolone in the basal excretion Following stimulation with ACTH however a part of the higher amount of 17-α hydroxy progesterone may be hydroxylated directly on 11-C and thus pregnanetriolone will be excreted in the urine In our family we found pregnanetriolone after stimulation with ACTH in the urine of the 2 healthy girls and of the parents The father however excreted pregnanetriolone in the basal urine (60 μg/d) We believe that in this family 4 of the 6 girls are homozygotes whereas the 2 others and the mother are heterozygotes [illegible] a partial 21 hydroxylase deficiency We think therefore that the estimation of pregnanetriolone in the urine following ACTH stimulation may be a method for the biochemical identification of symptomless heterozygous carriers of 21-hydroxylase deficiency

J R Guell Gonzalez, A Paramio-Rubial & B Delgado-Morales (Havana) *Male pseudohermaphroditism with internal bisexual genitals A report on two brothers*

The cases of two brothers with well-developed Mullerian ducts (tubes and uterus) together with structures derived from the Wolffian ducts and apparently normal external male genitalia and karyotype 46 XY are reported

Nacreous white testis with thickened albuginea prepubertal seminiferous tubules with sparse spermatogonia and interstitial tissue containing abundant fibroblastic elements were found There were no ovarian structures

We consider this condition as a syndrome of well defined clinical characteristics and with a recessive inheritance Its etiology is postulated as a genetic testicular defect by which there is a deficit in the production and/or release of the substance that provokes the involution of the Mullerian structures

We consider the removal of all internal genital structures together with the gonads to be the ideal treatment

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S B Pal & W Teller (Ulm) *Urinary 11 oxygenation index in children of various ages with and without adrenal disorders*

The urinary 11 oxygenation index is the ratio of the quantity of corticosteroids not oxygenated at 11 position of the steroid molecule such as pregnanetriol THS etc (abnormal metabolites) to that of corticosteroids oxygenated at 11 position of the steroid molecule like THE allo THF THF etc (normal metabolites) which are excreted in the urine The procedure for determination involves reduction of urine with sodium borohydride oxidation with sodium metaperiodate extraction with methylene dichloride and paper chromatography followed by the estimation of 2 steroid groups after Zimmermann reaction In 32 normal children aged between 3 days and 13 years, the mean 11 oxygenation index was 0 19 (range 0–0 4) and was the same irrespective of age In patients with adrenal disorders especially with congenital adrenal hyperplasia 11 oxygenation index rose above 1

References

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M Pierson, D Olive J Duheille & N Neimann (Nancy) *Paraneoplastic hyperthyreosis with thyroid antibodies in a case of infantile lymphosarcoma*

Clinical features of hyperthyreosis appeared in a girl aged 5 suffering from a mediastinal lymphoblastic lymphosarcoma

Biological investigations showed the usual patterns of excessive thyroid stimulation but no L A T S activity in the serum Thyroid antibodies in blood were detected especially anticytoplasmatic and antimitochondrial antibodies

Because the clinical situation was worsening treatment with synthetic antithyroid substances was started but no improvement in thyroid function could be obtained until antimiotic drugs were used to reduce the neoplastic process

This amelioration seemed to be strongly correlated with the regression of tumour lesions

Tentative studies were performed in order to determine whether the antibodies may be considered responsible for thyroid overstimulation

No conclusive arguments could be demonstrated

A Schwenk & U Steinicker (Koln Lindenthal) *Excretion of individual 11 deoxy 17 ketosteroids in a group of normal children and adolescents and in a comparative group suffering from endocrine disorders including adrenogenital syndrome applying the method of Treiber & Oertel (solvolysis and thin layer chromatography of dinitrophenylhydrazones)*

Quantitative assays of 17 ketosteroids in the diagnosis of endocrine disorders especially in the assessment of the treatment of the congenital adrenogenital syndrome using the method of Zimmermann and modifications thereof are liable to some error Therefore a method of analysis (described in this paper) more specific and precise and at the same time not as time consuming as other methods reported in the literature may be of interest The procedure employed by us (assaying 11 deoxy 17 ketosteroids only) is the following solvolysis of an aliquot of the 24 hour urine after De Paoli et al adding dinitrophenyl hydrazine in ethyl acetate to the residue then adding trichloroacetic acid in benzene thin layer chromatography of the residue dissolved

in chloroform on silicagel with chloroform/dioxane (94 6) with dehydroepiandrosterone androsterone and etiocholanolone at the same time scratching off the yellow-colored spots spectrophotometric assay at 335 370 and 405 nm using androsterone as standard With this method 24-hour urine specimens of 32 boys and 32 girls from a few days up to 16 years without endocrine disorders and also 20 boys and 21 girls ranging from infancy to 20 years with congenital adrenogenital syndrome other different endocrine disorders and disorders of somatic sexual development have been assayed

J J van der Werff ten Bosch A Bot & B M Goslings (Leiden) *The tricho-rhino-phalangeal syndrome*

R Wolter M Toppet & H Loeb (Brussels) *Plasma HGH and cortisol responses to insulin hypoglycemia after a 24 hour fast*

The plasma HGH and cortisol responses to insulin hypoglycemia after a 24-hour fast were studied in 25 normal children and compared with the responses found after a 12 hour fast in 60 normal children 0 1 U/kg of regular insulin was injected intravenously HGH was measured by double antibody radioimmunoassay with Wilhelmi HGH (HS 705A) as standard Plasma cortisol was measured by competitive protein binding radioassay

In comparison to the data found after a 12 hour fast the following facts were observed after a 24-hour fast

(*a*) Blood glucose was lower at fasting level (51 8 ± 9 2 mg/100 ml) and after 120 min (48 4 ± 8 9 mg/100 ml) but the minimum level was comparable (25 9 ± 9 9 mg/100 ml)

(*b*) Mean plasma HGH was consistently higher during the whole test The mean maximal value was 38 5 ± 15 0 ng/ml as compared with 17 4 ± 4 9 ng/ml

(*c*) Mean maximal values of plasma cortisol were slightly higher but the main effect seemed to be an increase in basal values

Paired insulin tolerance tests after a 12 hour and a 24 hour fast were studied in 24 children either normal or growth retarded Comparison of the plasma HGH maximal responses showed a positive correlation Hypopituitary children showed no plasma HGH increase In some children with responses below 6 ng/ml after a 12 hour fast a higher peak was observed after a 24-hour fast

Evidence is thus presented that insulin tolerance tests performed after a 24 hour fast induce higher plasma HGH responses This method is useful for the evaluation of pituitary HGH reserve Although fasting blood glucose was lower than normal tolerance was good in all the children possibly because minimal blood glucose values were comparable to those of the 12 hour fast tests

M Binoux M Th Pham Huu Trung M Gourmelen F Girard & P Canlorbe (Paris) *Plasma ACTH in adrenogenital syndrome*

A joint study of the corticotropin activity of plasma and the circulating cortisol level was undertaken in 39 children with the adrenogenital syndrome

Eighty three samples were taken between 9 and 10 a m without treatment they enabled us to determine the mean level of plasma ACTH as 0 51 mU/100 ml (range ≤0 10–2 3) or three times the norm Large variations deviating from this mean were observed from day to day from one patient to another and even in the same patient We were unable to correlate this with the degree of virilism the existence of sodium loss the length of the illness or the fact that the patient had been treated

Pituitary stimulation induced by metyrapone caused a considerable elevation in the plasma concentration of ACTH the level of which evaluated in 23 patients ranged from 0 59 mU/100 ml (range ≤0 10–2 2) to 3 44 mU/100 ml (range 1 2–11 0) This represents a value four times greater than in normal subjects having the same test

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BOOK REVIEWS

R J Robinson (ed) *Brain and early behaviour* Academic Press Inc London 1970 $15 00

The book is a compilation of lectures presented to the Study group on brain mechanisms of early behavioural development at the Ciba Foundation London February 1968

After a perspicuous introduction the content is divided in four main parts The fetus The infant—with subdivisions on physiological and behavioural studies development of sleep development of vision as well as learning and conditioning Primate studies Attempts at synthesis

Most papers are meritoriously ended with a conclusion or a summary after which there is a discussion on the topic The content of the papers is exhaustive with clear figures and tables throughout the book is a good documentation on early behaviour analysed from the viewpoint of the underlying brain mechanisms

Some reports must be specially mentioned The report by Tryphena Humphrey regarding postnatal repetition of fetal activity sequences excellently illustrates the avoiding or negative types of head movement in response to perioral stimuli as well as the positive reactions moving the area touched towards the stimulus the maturation of finger movements into grasping and the development of plantar reflexes The paper by F J Schulte et al concerns excitation inhibition and impulse conduction in spinal motor neurones in relation to conceptional age and body weight the neurophysiological mechanisms depend mainly on either structural maturation or the external and internal environment of the infant The increasing interest around different states of sleep and specially REM sleep has been documented by O Petre-Quadens et al They compare REM sleep in women at different periods of gestation and post partum with that found in relation to premature fullterm newborn and post mature normal babies REM sleep maximum occurs at the same conceptional age" in mothers and babies

The book is too specialized to be read and fully understood by pediatricians in general It pertains to the well-trained neuropeditrician as well as to developmental biologists and specially interested psychologists

O Eeg-Olofsson

Paul Lichtlen *Klinische Vektor Elektrokardiographie* Springer Verlag Berlin Heidelberg and New York 1969 235 pp illus DM 86 —

A vectorcardiogram (VCG) is a tridimensional display of all the resultant instantaneous electromotive forces generated during myocardial activity The vectorcardiogram is usually presented in the form of a loop which has components corresponding to those of the ordinary ECG to which it is an adjunction VCG has been widely used in adult cardiology in the detection of myocardial infection ventricular hypertrophy or intraventricular conduction disturbances but it has not been able to replace ECG which is a much easier way of recording the myocardial activity

This book in German gives a detailed analysis of the infarction diagnosis by VCG in relation to the coronary angiograms which are of a high quality In the demonstration of ventricular as well as atrial hypertrophy relations to hemodynamic and anatomical findings are shown Congenital heart diseases are represented by typical cases of atrial and ventricular septal defects Fallot transposition of the great arteries pulmonary stenosis and Ebstein The Frank system has been used and the loops are clearly demonstrated in connection to ordinary ECG and there are several very instructive and schematic figures

The book is useful for these working daily with ECG interpretation and it is also of value for those who want to learn the VCG method There is however only a limited experience in pediatric cardiology presented in the book and no comments of the possibilities of analysis by computer

Claes Thoren

LVP test performed in 9 patients did not induce any variation of cortisol Plasma ACTH rose from 0 49 (range 0 15–2 3) to 0 81 mU/100 ml (range 0 12–2 2) This represents an increase proportionately similar to the normal controls

The level of circulating ACTH measured in 6 patients at different times during the nycthemeral period varies in a cyclical fashion parallel to that of cortisol as in the normal state

These facts elucidate to some extent the pituitary adrenal regulation mechanisms in the adrenogenital syndrome

G B Forbes & D Schalch (Rochester) *An attempt to measure growth hormone production in neonates*

The standard method for estimating growth hormone (GH) production by the pituitary which involves a constant intravenous infusion of labelled hormone is obviously impractical for the neonate

The procedure of exchange transfusion involves the progressive dilution of the infant s blood with adult blood containing a low level of GH If two consecutive exchange transfusions are done in the same infant but at different rates one can set up a pair of simultaneous equations in this form $dQ/dt = PR - kQ - LR$ where Q is quantity of GH in the ECF, k the fractional degradation rate and LR the loss rate due to the procedure From measurements of GH and hematocrit in each aliquot of blood withdrawn, dQ/dt and LR are determined whereupon the equations can be solved for the two unknowns PR and k

After several attempts we were successful in the case of only one infant who met the important stipulations of (*a*) relatively quiet behavior, (*b*) absence of wide fluctuations in plasma GH and (*c*) a stable blood glucose level

The result was a production rate of 411 ng/min (0 6 mg/day) and a fractional degradation rate of 0 036/min (half life 19 min) both of which are close to those reported for adults

Hence the high level of plasma GH commonly found in neonates may not be due to either a high production rate by the pituitary or to slow degradation by peripheral tissues The most likely explanation is the small volume of body fluid available for dilution of the hormone in comparison with that of the child or adult

J R Guell Gonzalez E Alavez Martin B Arce Hidalgo A Navarro Lauten O Diaz Diaz & O M de Acosta Fernandez (Havana) *Congenital generalized lipodystrophy Report of six cases*

C G Bergstrand

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ANNOUNCEMENTS

SIR JOSEPH BARCROFT CENTENARY SYMPOSIUM ON FOETAL AND NEONATAL PHYSIOLOGY

A Symposium will be held by the British Physiological Society in Cambridge on 24-28 July 1972 to commemorate the centenary of the birth of Sir Joseph Barcroft The programme will contain communications from invited speakers together with free communications Sessions will include discussion periods covering Endocrinology Metabolism Respiration Circulation Placenta, the Nervous System and Parturition Participation will be limited to 250 active members of the Symposium

A Commemoration Dinner will be held in King's College Cambridge on 26 July 1972

Further information may be obtained from D P W Nathanielsz The Sir Joseph Barcroft Centenary Symposium Physiological Laboratory Cambridge England Requests for information should be accompanied by an indication of any intent to present a free communication reporting novel work together with a provisional title

THE AMERICAN PEDIATRIC SOCIETY, INC

The annual meetings of the American Pediatric Society the Society for Pediatric Research and the Ambulatory Pediatric Association will be held in the Sheraton Park Hotel Washington DC 22-26 May 1972 For information write to Charles D Cook M D (Secretary American Pediatric Society 333 Cedar Street New Haven Connecticut 06510) Robert E Greenberg M D (Secretary Treasurer Society fo Pediatric Research 12012 Compton Avenue Los Angeles California 90059) or Katherine S Lobach M D (Secretary Ambulatory Pediatric Association 1175 Morris Park Avenue Bronx New York 10461)

THE SECOND INTERNATIONAL BEILINSON SYMPOSIUM

The Second International Beilinson Symposium on the various faces of diabetes in juveniles (medical and rehabilitation aspects) is to take place in the vicinity of Tel Aviv from Oct 15 to Oct 20 1972

Further information can be obtained from Organizing Committee Symposium on the Various Faces of Diabetes in Juveniles (Medical and Rehabilitation Aspects) P O B 16271 Tel Aviv Israel

Acta Pædiat Scand 61 273–278 1972

CEREBRAL ARTERIO VENOUS DIFFERENCE OF ACETOACETATE AND D β HYDROXYBUTYRATE IN CHILDREN

B PERSSON G SETTERGREN and G DAHLQUIST

From the Department of Paediatrics Karolinska Institutet S t Gorans Hospital Stockholm Sweden

Studies in adult humans have demonstrated that following starvation for 5–6 weeks aceto acetate and D β hydroxybutyrate (ketone bodies) replace glucose as the predominant source of energy for the brain (17) A signifi cant cerebral arterio venous difference for ketone bodies has also been reported in adult humans after only 12–16 hours of fasting (7) The enzymatic prerequisites for ketone body utilization by the human brain (0–70 years) have recently been demonstrated on necropsy material (18)

A significant cerebral arterio-venous dif ference of ketone bodies which was propor tional to the arterial concentration has been described in young and adult rats (8) The cerebral arterio venous difference was three to four times higher at a given concentration in young as compared to adult rats (8) These observations and recent in vitro studies indi cate that ketone bodies may be of greater importance as an alternative substrate for glucose for the brain of growing rats as com pared to adult animals (2 3 5 9 11 19 24)

The rate of cerebral utilization of ketone bodies is apparently dependent on the activity of cerebral enzymes involved in ketone body catabolism and the amount of ketone bodies supplied to the brain per unit time At present there is limited knowledge about the role of ketone bodies as substrate for the human brain during development Elevated blood levels of ketone bodies have been reported in infants at birth and during the neonatal period in in fants who have received routine nursery care (20 21 22 23) Children 2–6 years of age have significantly higher overnight fasting values of D β hydroxybutyrate and acetoacetate than older children and adults (Persson unpub lished) and are more prone to develop keto nemia when fasting is prolonged (12) The present study deals with the cerebral arterio-venous difference of ketone bodies in children aged 6 weeks to 12 years

MATERIAL AND METHODS

Seven children aged 6 weeks to 12 years were studied in connection with surgical operations (Table 1) The indications for surgery and the length of fasting are also given in Table 1 All patients were given general anaesthesia after premedication with atropine and morphine depending on age (6) After an intravenous induction with thiopentone (3–4 mg/kg body weight) three had nitrous oxide (70% and relaxant) two had Neurolept type II (droperidol 0 3 mg/kg body weight and fentanyl 4 5 microgram/kg body weight in com bination with 50% nitrous oxide and oxygen) Two received methoxyflurane (0 15%) nitrous oxide (50%) and oxygen They were all intubated and artificially ventilated with a volume/time cycled ventilator (UR 70 Cameco Stockholm Sweden) After induction of anaesthesia catheters were inserted percutaneously into a radial artery and into an internal jugular vein During operation the patients were given a solution containing 0 9% glucose and 130 mE/l of sodium chloride intravenously The rate of infusion was 5 ml/ kg body weight/hour After operation they were given an intravenous infusion of 5% fructose and

Table 1 *Composition of material and indications for surgery*

Patient	Sex	Age	Diagnosis	Length of fasting (hours)	Number of arterio-venous samples	
					During anaesthesia	Awake
G E	F	6 weeks	Ductus arteriosus persistens Ventricular septal defect	6	4	10
K P	M	8 weeks	Pyloric stenosis	6	4	4
D S	M	1 $^{5}/_{12}$ years	Midline hernia (Status post omphalocele)	16	1	
A E	F	1 $^{11}/_{12}$ years	Atrial septal defect	12	3	3
M L	M	6 $^{7}/_{12}$ years	Pulmonary metastasis (Wilm s tumor)	12	8	8
L J	M	7 $^{7}/_{12}$ years	Anomalous coronary artery	8	8	4
A L	F	12 $^{4}/_{12}$ years	Pericarditis	12	1	2

5 glucose at a rate of 580-1 160 ml/m² body surface in 24 hours

Blood sampling

Paired blood samples (each 1 6 ml) were drawn into heparinized syringes during and after anaesthesia The number of paired samples are given in Table 1 The catheters were flushed with a dilute heparine 0 15 M sodium chloride solution The total amount of heparin administrated never exceeded 30-40 IE/kg body weight In four patients (G E K P M L and L J) four paired samples were taken during 10 min periods both during and after anaesthesia A 100 microliter aliquot of blood was immediately deproteinized with ice cold perchloric acid for later analysis of lactate and pyruvate (1) Part of the remaining blood sample was stored in ice water and centrifuged within 15 min Plasma was then separated and frozen for later analysis of glucose acetoacetate *D β* hydroxybutyrate glycerol and free fatty acids (FFA) Blood gas analyses were performed within 30 min The blood was stored in ice water until analysis

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pH base excess and Po were analysed using the Astrup technique and Radiometer equipment (Radiometer Copenhagen Denmark) Pco was calculated by interpolation Oxygen content was measured using

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K P	0 132±0 015	0 262±0 036	8 91±0 12	1 01±0 01	0 094±0 00	0 077±0 00
D S	0 415	0 694	9 39	1 79	0 146	—
A E	0 461[a] 0 336[b]	2 839 0 551	— —	2 11	0 091	—
M L	0 191±0 021	0 354±0 036	8 73±0 09	1 75±0 022	0 124±0 005	0 124±0 00
L J	0 210±0 007	0 605±0 010	5 77±0 15	2 21±0 08	0 103±0 007	0 088±0 00
A L	0 285	0 349	6 11	0 66	0 070	—
Adults[c]	0 042±0 019	0 082±0 054				

[a] Values obtained during hypothemia oesophageal temperature was 20 C These values are not included in calculation in Figs 1 and 2
[b] Values obtained 2 hours after anaesthesia in normothermia rectal temperature was 36 C
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the technique described by Linden et al (15) with the following minor modifications A microcathode oxygen electrode was used (Radiometer 5046 Copenhagen Denmark) A 100 microliter aliquot of blood was mixed anaerobically with 3 33 ml of a 0 4% potassiumferricyanide water solution (37 C) with a Po of 2 mmHg obtained by bubbling nitrous oxide through the solution Before calibrations and measurements the electrode was flushed with 1 ml of potassium ferricyanide solution and the sample was introduced into the electrode when the measuring instrument read 30 mmHg The same sample volume was used for calibration and measurement These procedures reduced the hysteresis of the electrode After each duplicate determination the electrode was recalibrated and the observed oxygen tensions were compensated for electrode drift With these modifications the reproducibility of the method was $\pm 0\ 053$ mM at a mean value of 4 1 mM oxygen/l blood Plasma glucose was determined by a hexokinase method (Boehringer Mannheim GmbH Germany) Plasma FFA was determined with a colorometric micromethod (13) $D\ \beta$ hydroxybutyrate acetoacetate and glycerol were analysed by enzymatic fluorometric techniques (14 22) The analytical errors were for glucose $\pm 1\ 22$ for FFA $\pm 2\ 19$ for acetoacetate $\pm 0\ 69$ for $D\ \beta$ hydroxybutyrate $\pm 0\ 50$ for glycerol $\pm 0\ 56$ for lactate $\pm 1\ 75$ and pyruvate $\pm 2\ 72\%$ respectively

RESULTS

The initial arterial concentrations of acetoacetate $D\ \beta$ hydroxybutyrate glucose lactate pyruvate glycerol FFA P_O P_{CO_2} and base excess are shown in Table 2 The correspond

FFA (mM)	PO (mmHg)	PCO (mmHg)	B E (mE/l)
1 66 ± 0 04	183	4? ± 3 0	−2 5
1 31 ± 0 0	94 ± 4	6 ± 0 2	−8
—	—	4.	−6 5
—	180	0	−9 5
1 09 + 0 00?	103	38 5 ± 1 4	−9 0
–	130 ± 1	25 5 + 2.2	−6 5
—	90	33	−6 0

ing initial cerebral arteriovenous differences for acetoacetate $D\ \beta$ hydroxybutyrate glucose and oxygen content are given in Table 3 There was no significant cerebral arterio venous difference for lactate pyruvate FFA and glycerol The mean ($\pm$S E) cerebral arterio venous differences ($n = 19$) for glucose and oxygen were $0\ 49 \pm 0\ 08$ and $2\ 38 \pm 0\ 17$ mM respectively during anaesthesia The corresponding mean differences ($n = 19$) after anaesthesia were $0\ 52 \pm 0\ 10$ and $2\ 03 \pm 0\ 08$ mM respectively There was a significant correlation ($p < 0\ 001$) between the arterial plasma concentration of acetoacetate + $D\ \beta$ hydroxy butyrate and the cerebral arterio venous difference (Fig 1) This relationship did not seem to be influenced by anaesthesia

When the patients were divided into two age groups one consisting of the four children below 2 years of age and the other of the remaining three older children the equations of the regression lines were $y = 0\ 002 + 0\ 137x$ ($n = 26$ $r = 0\ 84$) and $y = 0\ 008 + 0\ 092x$ ($n = 31$) ($r = 0\ 73$) respectively (x = arterial concentration of acetoacetate + $D\ \beta$ hydroxybutyrate y = arterio venous difference of acetoacetate + $D\ \beta$ hydroxybutyrate) The slopes of the regression lines were not statistically different ($p > 0\ 05$) Fig 2 shows the regression lines for the arterial plasma concentration of acetoacetate and $D\ \beta$ hydroxybutyrate versus the cerebral arterio-venous differences The 99 intervals of confidence for the slopes of the lines are separate

DISCUSSION

The initial arterial concentrations of acetoacetate and $D\ \beta$ hydroxybutyrate in the children studied were significantly higher than overnight fasting values of adults (Table 2) The initial ketone body levels in the present study were however quite comparable to those determined in healthy non hospitalised children of 2–6 years of age after an over night fast (Persson unpublished)

During anaesthesia and operation the ar

Table 1 *Composition of material and indications for surgery*

Patient	Sex	Age	Diagnosis	Length of fasting (hours)	Number of arterio-venous samples	
					During anaesthesia	Awake
G E	F	6 weeks	Ductus arteriosus persistens Ventricular septal defect	6	4	10
K P	M	8 weeks	Pyloric stenosis	6	4	4
D S	M	$1^{5}/_{12}$ years	Midline hernia (Status post omphalocele)	16	1	
A E	F	$1^{11}/_{12}$ years	Atrial septal defect	12	3	3
M L	M	$6^{7}/_{12}$ years	Pulmonary metastasis (Wilm s tumor)	12	8	8
L J	M	$7^{7}/_{12}$ years	Anomalous coronary artery	8	8	4
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[a] Values obtained during hypothermia oesophageal temperature was 20 C These values are not included in calculatio in Figs 1 and 2

[b] Values obtained 2 hours after anaesthesia in normothermia rectal temperature was 36 C

[c] Normal overnight fasting values for acetoacetate and *D β* hydro[illegible] for comparison (2-)

gesting that in children and adults a measurable cerebral arterio venous difference occurs above an arterial concentration of approximately 0 25 mM (acetoacetate + D β hydroxybutyrate) The significant difference in slopes of the regression lines for acetoacetate and D β hydroxybutyrate given in Fig 1 was in accordance with observations in rats (8) and compatible with the findings in dogs of a more rapid penetration of acetoacetate into the cerebral spinal fluid than of D β hydroxy butyrate (25)

The difference in slopes of regression lines (arterial concentrations of acetoacetate + D β hydroxybutyrate versus the cerebral arterio-venous difference) between children below 2 years and above 2 years of age—though not statistically so—might indicate a higher cerebral arterio venous difference at a given arterial concentration in the youngest group of children This observation could be in accordance with the findings of a higher capacity to utilize ketone bodies in rat brain during the period of myelinization (8 9 19) The maximum rate of myelinization in humans occur around the age of six months thereafter the rate declines progressively and is slow after the age of two years (4) The mean initial cerebral arterio-venous difference for glucose was in agreement with reported values in adults (16) whereas the corresponding difference in oxygen was slightly lower than those found in adults (16)

It should be noted however that these values were obtained during anaesthesia in contrast with those of the adults who were examined awake Further studies are needed to determine the quantitative importance of ketone bodies in relation to glucose as substrate for the developing human brain Variations in cerebral arterio venous differences at a given arterial concentration do not necessarily reflect differences in cerebral uptake since the cerebral blood flow might vary with age (10) Furthermore variations in depth of anaesthesia and of arterial carbon dioxide tension do influence cerebral blood flow

SUMMARY

Cerebral arterio-venous differences of acetoacetate D β hydroxybutyrate glucose glycerol FFA lactate pyruvate and oxygen were studied in seven children aged 6 weeks–12 years during and after anaesthesia Significant arterio-venous differences were found for glucose oxygen and ketone bodies A significant correlation between the arterial concentrations of acetoacetate plus D β hydroxybutyrate and their cerebral arterio venous differences was demonstrated ($p < 0\ 001$) The cerebral arterio-venous difference of acetoacetate was significantly higher than for D β hydroxybutyrate at the same arterial concentrations The results indicate a greater cerebral arterio-venous difference of acetoacetate and D β hydroxybutyrate at a given concentration as compared to reported values in adults after a comparable length of fasting The cerebral uptake of ketone bodies was not determined since cerebral blood flow was not measured in the present study

ACKNOWLEDGEMENT

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Table 3 *Cerebral arterio venous differences for ketone bodies glucose and oxygen during anaesthesia*

Patient	Acetoacetate (mM)	D β Hydroxybutyrate (mM)	Glucose (mM)	Content O_2 (mM)
G E	−0 102±0 010	−0 064±0 015	−0 79±0 33	−2 34±0 34
K P	−0 026±0 005	−0 027±0 008	−0 50±0 05	−2 31±0 13
D S	−0 025	−0 012	—	—
A E	−0 031[a] −0 081[b]	−0 450 −0 093	— —	— —
M L	−0 004±0 000	−0 007±0 000	−0 32±0 14	−1 27±0 07
L J	−0 022±0 008	−0 063±0 010	−0 42±0 13	−3 09±0 21
A L	−0 031	−0 006	—	—

[a] Values obtained during hypothermia
[b] During normothermia 2 hours after anaesthesia

terial levels of acetoacetate and *D β* hydroxybutyrate remained essentially unchanged but when the caloric supply was increased during the post operative period the arterial levels declined significantly The cerebral arterio-venous differences were thus determined at different arterial concentrations of ketone bodies in some patients (Table 1)

The present study has clearly demonstrated that children, like adults have a significant relationship between arterial concentrations of acetoacetate and *D β* hydroxybutyrate and the cerebral arterio venous differences This indicates a correlation between cerebral uptake of ketone bodies and the amount supplied to the brain per unit time

At comparable arterial concentrations of total ketone bodies (acetoacetate+*D β* hydroxybutyrate) the arterio venous difference seems to be greater in the present study than that reported in adults (7) Our results and those reported in adults show that the intercept of the χ axis of the regression lines was approximately the same sug

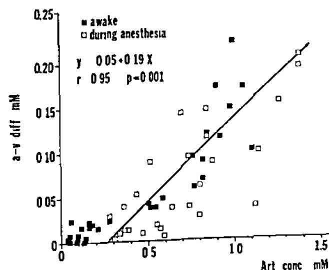


Fig 1 Arterial concentration of acetoacetate plus *D β* hydroxybutyrate versus their cerebral arterio venous difference

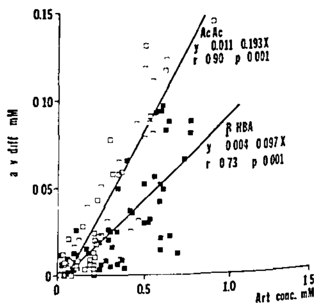


Fig 2 Arterial concentration of acetoacetate and *D β* hydroxybutyrate versus the cerebral arterio-venous difference

Acta Pædiat Scand 61 279–284 1972

EFFECT OF PROTEIN LOADING ON BLOOD PHENYLALANINE LEVELS IN NEWBORN INFANTS

JULIAN L. BERMAN ROSITA S PILDES and CHARLES M BLUMENFELD

From the Departments of Pediatrics Chicago Medical School University of Illinois the Division of Pediatrics Cook County Hospital Chicago and the Department of Pathology Sutter Community Hospital Sacramento California USA

Screening tests (1 2) for the presence of ab normal concentration of serum phenylalanine have made the detection of phenylketonuria (PKU) highly successful in the newborn (3 4 5) Although tests were designed for 100% detection some failures have been reported recently (6 7) A portion of these failures may be due to a slower than usual rise in the blood phenylalanine the usually accepted normal value being <4 mg/100 ml at the time of screening (7) Two recent reports have noted a discrepancy in the sex ratio of phenyl ketonurics detected in screening programs (8 9) The number of males to females was 2 1 instead of the expected 1 1 ratio This dis crepancy may have been due to a sampling error (10) or to a number of other causes (11–14)

The present study was designed to raise transiently the blood phenylalanine in new borns potentially heterozygotic for PKU If this could be accomplished it would be likely to do so in the phenylketonuric child as well Possible PKU screening failures (15) would be detected earlier if they were due to a slower than usual rise in blood phenylalanine In ad dition several types of variants or atypical cases might also be disclosed

Two related phenylalanine loading studies were done The first hypothesized that normal newborns would not have an elevated blood phenylalanine value (>4 mg/100 ml) after a small increase in dietary phenylalanine The second to see if this were true for newborns potentially heterozygotic for PKU Our results indicated that neither had a transient rise

METHODS

Normal newborns were given a protein (phenyl alanine) load in the form of a proprietary milk (Olac) Evaporated Milk and Similac served as controls Evaporated Milk was used because it is our nursery s usual formula and Similac becau e of its lower protein content (16 Table 1) Serial blood phenyl alanine and tyro ine determinations were compared between and within each group

Five hundred and ninety infants were included in the first study and placed on one of 4 milk formulas All formulas were prepared from their liquid forms and used in the following dilutions Olac with and without the addition of Vitamin C (ascorbic acid) 20 cal/30 cc Similac 20 cal/30 cc and Evaporated Milk 15 cal/30 cc Since transient tyrosinemia of the new born (partially due to low ascorbic acid levels) can ef fect the level of blood phenylalanine (17 18) one group was placed on regular Olac (no Vitamin C) while another had additional 50 mg of ascorbic acid added to the 9 00 a m bottle

A newborn was included in the study if he was the product of a normal full term vaginal hospital delivery and completely well at the time of admission to the study All infants weighed >2 500 g and were between 38 and 41 weeks gestation The infants were placed at random in two preselected nurseries and the entire nursery was placed on one of the designated formulas Blood specimens were drawn in the morning from the heel within 24 hours of birth *prior to the* onset of a milk feeding (control) A second sample was obtained 60–72 hours later

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Table 1 *Phenylalanine protein and calorie content per 30 cc of milk (16)*

Milk	Human	Cow s	Olac	Evaporated	Similac
Dilution	—	—	1 1	1 2	1 1
Phenylalanine mg	19	51	54	36	27
Protein g	0 38	1 00	1 00	0 70	0 52
Calories	20	20	20	15	20

Each group was originally intended to include 100–200 infants In some instances the numbers were reduced because of technical nursery problems This is exemplified by the Olac with Vitamin C Group in which several infants developed a non specific diarrhea during the course of the study However it was felt that sufficient numbers of infants had been included to justify the termination of this study group A total of 590 infants were placed in one of four groups 180 Olac (Group A) 88 Olac with Vitamin C (Group B) 199 Evaporated Milk (Group C) and 123 Similac (Group D) The numbers were further decreased due to the exclusion of an infant if any of the following were present inadequate paired specimens early discharge or subsequent illness The final number (457) included 106 Olac 72 Olac with Vitamin C 169 Evaporated Milk and 110 Similac

The second study consisted of 16 infants whose mothers had previously given birth to a child with PKU or Hyperphenylalaninemia (Group E) Two infants were excluded since one was found to have true PKU and the other was a half sib Samples for phenylalanine and tyrosine were usually collected from the cord blood prior to (<24 hours) and at the onset of feeding (24 hours) and daily thereafter for 3 days A subsequent phenylalanine tolerance test to verify heterozygosity was performed within the first 2 months as described by Cunningham et al (19)

Those subsequently found to be normal were termed Group E_1 and those found to be heterozygotic were termed Group E_2 All blood samples for phenylalanine determinations were tested in one or more of three laboratories The samples remaining in Chicago (Laboratory 1 JLB) were separated from the erythrocytes and the plasma frozen and analyzed at a later date by the method of McCaman & Robbins (2) The normal value for this laboratory was 0 9 to 2 5 mg/100 ml

Triplicate samples were sent Air Mail to the other laboratories Laboratory 2 (CMB) for Guthrie analysis (1) (normal 2–4 mg/100 ml) and Laboratory 3 (CMB) for densitometric phenylalanine evaluations (6) Blood tyrosine (Laboratory 1) was determined by the method of Wong et al (20) All blood determinations were analysed without prior knowledge as to the milk groupings of the infants

All comparisons of phenylalanine and tyrosine levels were calculated from paired samples from the same individual The significance of difference of the means was determined using Student s *t* test

RESULTS

A significant rise in the blood phenylalanine at 60–72 hours was common to all groups tested ($p < 0\ 02$) by the fluorometric method (Table 2) No significant differences were noted between groups The Guthrie and densitometry methods showed no difference between the control (first sample) and second samples (Table 2) Significant differences were sometimes found between Group D (Similac) and the others at the 60–72 hour period by both of these methods however these differences were also found in the control samples No differences in blood tyrosine were noted between the four groups although a significant rise was noted at 60–72 hours in Group C (Table 3) There were no significant differences between males and females in any of

Table 2 *Blood phenylalanine levels (means ±S D) in newborns using the fluorometric Guthrie and densitometry methods*

		*Fluorometric method			**Guthrie method			**Densitometry method		
Group	Milk	*n*	Control (mg/100 ml)	60–72 hours (mg/100 ml)	*n*	Control (mg/100 ml)	60–72 hours (mg/100 ml)	*n*	Control (mg/100 ml)	60–72 hours (mg/100 ml)
A	Olac	106	1 57±0 49	1 98±0 56	69	2 27±0 69	2 63±0 56	61	2 58±0 94	2 73±1 03
B	Olac+ C	72	1 63±0 48	2 13±0 51	52	2 24±0 87	2 20±0 96	58	2 39±0 53	2 28±0 91
C	Evaporated	169	1 73±0 55	2 15±0 64	155	2 13±0 60	2 06±0 65	104	2 07±0 60	1 89 52
D	Similac	110	1 52±0 60	2 14±0 85	107	2 66±0 53	2 60±0 53	76	2 63±0 65	2

* Control vs 60–72 hours $p < 0\ 02$
** Control vs 60–72 hours $p > 0\ 10$

Table 3 *Blood tyrosine (mean ±S D) in newborn infants*

Group	n	Control (mg/100 ml)	60–72 Hours (mg/100 ml)
A	13	2 49±1 50	2 16±1 27
B	38	1 94±0 74	2 63±1 54
C	57	2 32±0 69	3 76±1 15
D	57	2 38±0 68	2 89±1 08

$p<0\,02$ Control vs 60–72 hours

the comparisons The number of males was significantly less than females in Group B and greater in Group D The total numbers of males and females were similar when all groups were combined (225 to 232)

Two infants in Group C and one in Group D had blood phenylalanine levels of >4 mg/100 ml (fluorometric) prior to the onset of milk feeding After 60–72 hours these numbers increased to 3 infants in Group C and 2 in Group D Each of these patients was tested a third time within the next few days and found to be within normal limits No significant rise was seen in either E_1 or E_2 though Group E_1 had less of a rise than did Group E_2

The results of a 4 hour phenylalanine test in 13 normal infants (Group F) and 14 infants in Group E (all of whom were 1 to 2 months of age) are shown in Table 4 The phenylalanine values tend to rise less than those seen in children or adults (7) It should be noted that the high range of the normal newborns at 1 2 and 4 hours was accounted for by one infant This infant may represent a heterozygotic state but it was not possible to retest that child There were no significant differences between Groups E_1 and F but marked differences at all hours between E_2 and E_1 and E_2 and F

Table 5 shows the individual blood phenylalanine levels for Group E These infants were siblings of patients known to have classical PKU except newborns 3 and 13 who are siblings of a patient with transient hyperphenylalaninemia Newborns 2 3 7 8 11 13 and (probably) 1 have normal curves Newborns 4 5 6 9 10 12 and 14 have results compatible with those who are heterozygotic Table 6 shows that none of these children including the probable heterozygotes increased above 3 6 mg/100 ml at 60–72 hours except for newborn 14 who had a level of 4 3 mg/100 ml at 72 hours This patient had a transient tyrosinemia of the newborn which decreased with the administration of ascorbic acid Patient 8 had high blood tyrosine levels at 24 48 and 72 hours of age reaching a maximum of 19 5 mg/100 ml at 72 hours which spontaneously reverted to normal at 2 weeks of age Both infants were full term and of uncomplicated pregnancies

DISCUSSION

By means of the fluorometric technique a significant rise in blood phenyalanine levels was demonstrated during the first 60–72 hours of life in normal full term newborn infants

Table 4 *Phenylalanine tolerance tests (19) in normal newborns and infants potentially heterozygous for phenylketonuria*

Hour	Newborns (13) (normal Group F)		Potential heterozygotes (7) (normal Group E_1)		Potential heterozygotes (7) (heterozygotic Group E_2)[b]	
	Mean±S D	Range	Mean±S D	Range	Mean±S D	Range
0	1 20±0 21	0 8– 1 5	1 36±0 22	1 1–1 7	2 86±0 89	1 7– 3 9
1	4 54±1.29	3 2– 6 9	4 39±2 19	2 1–8 1	7 15±0 91	5 4– 8 5
2	5 75±2 06	3 6–11 6	4 71±1 29	3 0–6 4	9 44±1 03	8 6–11 5
4	3 47±1 13	2 0– 6 2	3 43±0 82	2 5–4 6	9 30±3 04	6 2–15 1

$p>0\,10$ E vs F
[b] $p<0\,001$ E_2 vs F

Table 1 *Phenylalanine protein and calorie content per 30 cc of milk (16)*

Milk	Human	Cow s	Olac	Evaporated	Similac
Dilution	—	—	1 1	1 2	1 1
Phenylalanine mg	19	51	54	36	27
Protein g	0 38	1 00	1 00	0 70	0 52
Calories	20	20	20	15	20

Each group was originally intended to include 100–200 infants In some instances the numbers were reduced because of technical nursery problems This is exemplified by the Olac with Vitamin C Group in which several infants developed a non specific diarrhea during the course of the study However it was felt that sufficient numbers of infants had been included to justify the termination of this study group A total of 590 infants were placed in one of four groups 180 Olac (Group A) 88 Olac with Vitamin C (Group B) 199 Evaporated Milk (Group C) and 123 Similac (Group D) The numbers were further decreased due to the exclusion of an infant if any of the following were present inadequate paired specimens early discharge or subsequent illness The final number (457) included 106 Olac 72 Olac with Vitamin C 169 Evaporated Milk and 110 Similac

The second study consisted of 16 infants whose mothers had previously given birth to a child with PKU or Hyperphenylalaninemia (Group E) Two infants were excluded since one was found to have true PKU and the other was a half sib Samples for phenylalanine and tyrosine were usually collected from the cord blood prior to (<24 hours) and at the onset of feeding (24 hours) and daily thereafter for 3 days A subsequent phenylalanine tolerance test to verify heterozygosity was performed within the first 2 months as described by Cunningham et al (19)

Those subsequently found to be normal were termed Group E_1 and those found to be heterozygotic were termed Group E_2 All blood samples for phenylalanine determinations were tested in one or more of three laboratories The samples remaining in Chicago (Laboratory 1 JLB) were separated from the erythrocytes and the plasma frozen and analy ed at a later date by the method of McCaman & Robbins (2) The normal value for this laboratory was 0 9 to 2 5 m / 100 ml

Triplicate samples were sent Air Mail to the other laboratories Laboratory 2 (CMB) for Guthrie analysis (1) (normal 2–4 mg/100 ml) and Laboratory 3 (CMB) for densitometric phenylalanine evaluations (6) Blood tyrosine (Laboratory 1) was determined by the method of Wong et al (20) All blood determinations were analysed without prior knowledge as to the milk groupings of the infants

All comparisons of phenylalanine and tyrosine levels were calculated from paired samples from the same individual The significance of difference of the means was determined using Student s *t* test

RESULTS

A significant rise in the blood phenylalanine at 60–72 hours was common to all groups tested ($p<0\ 02$) by the fluorometric method (Table 2) No significant differences were noted between groups The Guthrie and densitometry methods showed no difference between the control (first sample) and second samples (Table 2) Significant differences were sometimes found between Group D (Similac) and the others at the 60–72 hour period by both of these methods however these differences were also found in the control samples No differences in blood tyrosine were noted between the four groups although a significant rise was noted at 60–72 hours in Group C (Table 3) There were no significant differences between males and females in any of

Table 2 *Blood phenylalanine levels (means ± S D) in newborns using the fluorometric Guthrie and densitometry methods*

Group	Milk	*Fluorometric method			**Guthrie method			**Densitometry method		
		n	Control (mg/100 ml)	60–72 hours (mg/100 ml)	*n*	Control (mg/100 ml)	60–72 hours (mg/100 ml)	*n*	Control (mg/100 ml)	60–72 hours (mg/100 ml)
A	Olac	106	1 57±0 49	1 98±0 56	69	2 27±0 69	2 63±0 56	61	2 58±0 94	2 73±1 03
B	Olac + C	72	1 63±0 48	2 13±0 51	52	2 24±0 87	2 20±0 96	58	2 39±0 53	2 29±0 91
C	Evaporated	169	1 73±0 55	2 15±0 64	155	2 13±0 60	2 06±0 65	104	2 07±0 60	1 89±0 52
D	Similac	110	1 52±0 60	2 14±0 85	107	2 66±0 53	2 60±0 53	76	2 63±0 65	2 42±0 63

* Control vs 60–72 hours $p<0\ 02$
** Control vs 60–72 hours $p>0\ 10$

high blood tyrosine levels (4–5 mg/100 ml) which is consistent with the findings of Kretchmer et al (23) These investigators have shown that the tyrosine oxidizing system in full term humans is 3–8 times less than adults It would seem reasonable that the phenylalanine hydroxylase system would also be relatively immature Berman (24) has measured phenylalanine hydroxylase in newborn and fetal rats These animals have much less activity than do normal newborn rats who are a few days old Nevertheless the phenylalanine intake can be doubled in normal and heterozygotic human infants without causing a positive PKU screening test

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Table 5 *Phenylalanine tolerance tests (mg/100 ml) in newborn infants whose siblings have phenylketonuria or hyperphenylalaninemia*

Patient	Sex	0	1 hr	2 hr	4 hr
1	M	1 5	3 4	5 5	4 6
2	M	1 1	8 1	4 5	2 5
3	M	1 2	3 9	5 3	2 9
4[a]	M	3 6	7 6	8 7	8 0
5[a]	F	2 3	7 3	9 8	6 6
6[a]	F	1 7	5 4	8 6	6 2
7	M	1 7	3 9	5 3	4 2
8	F	1 2	2 1	3 0	2 7
9[a]	M	3 8	7 0	8 8	8 5
10[a]	F	3 9	8 5	11 5	10 7
11	M	1 5	6 7	6 4	3 9
12[a]	F	2 2	7 1	9 7	15 1
13	M	1 3	2 6	3 0	3 2
14[a]	M	2 5	7 2	9 0	10 0

[a] Abnormal

The rise was not seen when the same specimens were analysed by densitometry or the Guthrie methods, however, these techniques are less precise than the fluorometric method This increase was present whether the infants were given Olac Olac with Vitamin C Evaporated Milk or Similac There were no differences between males and females, though in two of the groups the male/female ratio was abnormal probably due to the small numbers in each of these groups Hambræus & Wranne (21) reported a decrease in phenylalanine in full term breast fed infants during the first

Table 6 *Blood phenylalanine levels (mg/100 ml) in newborn infants whose siblings have phenylketonuria or hyperphenylalaninemia*

Feeding begun 24 hours after birth

Patient	Cord	< 24 hr	24–30	44–52	60–72
1	1 3	1 3	1 5	1 5	1 3
2	1 5	2 1	2 1	1 6	1 3
3	—	—	1 6	—	2 0
4	2 4	2 5	3 2	2 4	2 5
5	2 1	1 8	1 6	1 6	3 6
6	1 6	1 3	1 6	1 6	1 8
7	2 0	—	1 7	1 2	2 2
8	1 7	1 7	1 5	1 9	2 6
9	2 4	1 4	2 1	1 9	2 0
10	3 0	2 6	2 4	2 5	3 0
11	1 2	1 2	1 2	2 4	2 4
12	—	1 8	1 2	1 4	2 0
13	—	1 6	1 5	2 2	1 2
14	2 7	1 7	2 1	1 7	4 3

5 days of life Although the fluorometric method was used only 23 infants were studied

Eight to 9 infants (66%) of the 14 potential heterozygotes (Group E) were expected to demonstrate a phenylalanine tolerance test compatible with the carrier state since PKU is a recessive disease Seven infants were observed to be heterozygous which is less than the expected though others might well have been heterozygous Only one of the 14 infants developed a blood phenylalanine level >4 0 mg/100 ml This infant had a transient tyrosinemia of the newborn This infant and a second whose level was next highest (3 6 mg/100 ml at 72 hours) were later shown to be carriers We can therefore assume that infants who are carriers for PKU do not cause false positive screening tests

These results do not support the hypothesis that a protein load would better detect a child with PKU who might be undetected due to an abnormally slow rise in blood phenylalanine Heterozygotic infants do rise somewhat higher than non heterozygotic infants and begin their rise at a higher level but these results were not significant

The study does provide data concerning the reports of a sex ratio difference in children with PKU There was no differential rise between the sexes in any of the groups tested, and thus sex differences cannot be attributed to a differential rise in blood phenylalanine

No differences were noted between milks in the first study when the phenylalanine was analysed by the fluorometric method and compared at 60–72 hours Similar results were found by Blumenfeld et al in an uncontrolled study of approximately 600 infants (22) In some instances using the densitometric and Guthrie techniques significant differences were noted between Olac and the other milks However the values were also higher in the control samples These findings re emphasize the importance of precise techniques when mean differences are small as well as the need for proper controls

Several of the infants studied had slightly

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Four hundred and fifty seven normal infants and 14 infants who were potentially heterozygotic for phenylketonuria were placed on Olac feeding during the first 60–72 hours of milk feeding This doubled their phenylalanine intake but did not raise their blood phenylalanine levels to greater than 4 mg/100 ml No differences were seen when various milks were compared Heterozygous infants for PKU were not significantly different from normal No sex differences were found and it was not possible to improve the detection of PKU using these methods

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9	2 4	1 4	2 1	1 9	2 0
10	3 0	2 6	2 4	2 5	3 0
11	1 2	1 2	1 2	2 4	2 4
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DISPLACEMENT OF ALBUMIN BOUND BILIRUBIN BY FATTY ACIDS

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Kernicterus may be precipitated in icteric newborns by displacement of bilirubin from binding to albumin by various drugs (11 14 15 17) Several investigators (12 16 19 20) have considered the possibility that fatty acids might have a similar effect competing with bilirubin for the binding sites on albumin Non-esterified fatty acids are bound reversibly to albumin (8 18) and are present in the blood plasma of newborn children in relatively high concentrations (6 13) The present paper deals with displacement of bilirubin by fatty acids from its binding to human plasma albumin measured by a sensitive technique recently described by Jacobsen & Fedders (10) based on determination of the velocity of oxidation of bilirubin with peroxidase and ethyl hydroperoxide

MATERIAL AND METHODS

Bilirubin and linolenic acid (cis 9 *cis* 12 *cis-15* octadecatrien acid) were obtained from Sigma (both Sigma grade) as well as horse radish peroxidase (*type I*) Lyophilized human plasma albumin was from KABI Sweden and was defatted with charcoal according to the method of Chen (4) Ethyl hydroperoxide was supplied by Ferrosan palmitic acid by Fluka and oleic acid by Merck

Samples of venous blood were collected from icteric newborns prior to exchange transfusion The serum was kept in the refrigerator and was used within 24 hours

Displacement of bilirubin from binding to albumin revealed by an increased *rate of oxidation* with peroxidase and ethyl hydroperoxide was determined as described by Jacobsen & Fedders (10) The principle of the method is as follows When peroxidase is added to a solution of bilirubin and albumin the two proteins will compete for the pigment The distribution of bilirubin will be strongly in favour of albumin (The dissociation constant of bilirubin albumin 1st site is about 10 M (9) while the Michaelis constant for bilirubin and horse radish peroxidase is 10 M (1)) When a substance is added capable of displacing bilirubin from its binding to albumin more of the pigment will be bound to peroxidase The velocity of the enzymatic oxidation of bilirubin is proportional to the amount of bilirubin bound to the enzyme and hence an increase of the velocity can be taken as a measure of the displacement of bilirubin caused by the substance added

Oxidation was performed at pH 7 4 37 in phosphate buffer 66 mM with EDTA 10 mM The reaction mixture consisted of 10 vols. of serum and 2 vols of the other ingredients. The procedure was facilitated by measuring the bilirubin concentrations by the acetone method (7) rather than by chloroform extraction as previously 100 μl samples of the reaction mixture were added to 2.5 ml acetone After centrifugation the extinction of the supernatant was measured in a Unicam SP 800 spectrophotometer at 455 nm 1 cm cell The millimolar extinction coefficient of bilirubin in acetone was found to be 54

The concentration of unconjugated bilirubin in serum was similarly determined by the acetone method

Non-esterified fatty acids were titrated acidimetrically according to Dole (5)

RESULTS

In a solution of pure albumin with added bilirubin at pH 7 4 (Fig 1) a low rate of oxidation is found when the molar ratio of bilirubin/albumin is 0 5 The oxidation rate increases 50 times when the amount of bilirubin is increased to 1 8 mol per mol albumin

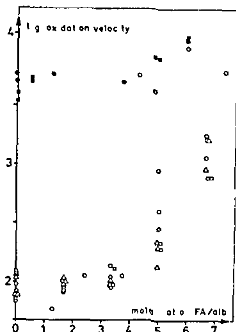


Fig 1 Human plasma albumin solution (4% 0 6 mM) with bilirubin and fatty acid added in alkaline solutions pH = 7 4 The logarithm of the initial velocity of oxidation of bilirubin with peroxidase and *ethyl hydroperoxide divided by the enzyme concentration* (ordinates min⁻¹) as a function of the fatty acid/albumin molar ratio ○ Palmitate bilirubin/albumin 0 5 ● Palmitate bilirubin/albumin 1 8 □ Oleate bilirubin/albumin 0 5 ■ Oleate bilirubin/albumin 1 8 △ Linolenate bilirubin/albumin 0 5

Addition of fatty acids until 4 mol per mol albumin causes no demonstrable increase of oxidation rate This is found equally with palmitate oleate and linolenate At higher fatty acid concentrations it is seen that oxidation rates increase considerably in case of the low bilirubin/albumin ratio 0 5 while a slight increase is observed at the high ratio 1 8 At high fatty acid concentrations the reproducibility of the oxidation rate measurement is very poor *This is probably due to precipitation of* bilirubin (3)

Sera from icteric newborns (Fig 2) without addition of bilirubin show increased rates of oxidation at concentrations of palmitate above 2 mM With an average concentration of plasma albumin of 0 5 mM (19) this corresponds to 4 mol fatty acid per mol albumin, in good agreement with the findings for the pure albumin solution (Fig 1)

A different pattern is observed with normal

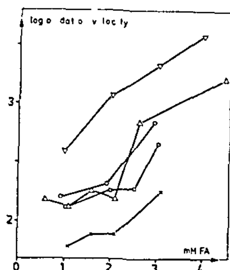


Fig 2 Blood sera from five icteric newborns with varying concentrations of palmitate added (abscissa) The left point from each specimen shows the native concentration of non esterified fatty acids Ordinates as *in Fig 1 The concentrations of unconjugated* bilirubin in the five specimens ▽ 0 26 mM (15 mg/100 ml) ○ 0 21 mM (12 mg/100 ml) △ 0 16 mM (10 mg/100 ml) □ 0 10 mM (6 mg/100 ml) × 0 07 *mM (4 mg/100 ml)*

adult sera after addition of approximately 0 5 mol bilirubin per mol albumin (Fig 3) No consistent change of oxidation rate is seen even with more than 4 mM palmitate which *is about 6 mol fatty acid per mol albumin*

DISCUSSION

Starinsky & Shafrir (19) have studied displacement of bilirubin from human plasma albumin to Sephadex by gel chromatography after ad

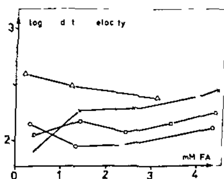


Fig 3 Blood sera from four adults with added bilirubin 0 3 mM (17 mg/100 ml) and with varying concentrations of palmitate (abscissa) Ordinates as in Fig 1

dition of linoleate (cis 9 cis-12-octadecadien acid) With 1 8 mol bilirubin per albumin they find no displacement of bilirubin when the amount of fatty acid is below 4 mol while gross displacement occurs when larger amounts of linoleate are added With 0 5 mol bilirubin displacement is not seen until about 6 to 7 mol fatty acids is added per mol albumin Since in the newborn child the molar ratio of fatty acid/albumin ranges from 1 07 to 2 37 these authors suggest that fatty acid concentrations do not reach bilirubin displacing levels It may be argued however that the Sephadex method is unable to demonstrate a slight degree of displacement which might take place with small concentrations of fatty acids and could be clinically important This is seen from the fact that Starinsky & Shafrir find little displacement of bilirubin to Sephadex when the molar ratio of bilirubin to albumin is 1 8 (without fatty acid) The concentration of free nonalbumin bound bilirubin in this solution is of the order of one hundred times higher than at the molar ratio 0.5 (9) This difference of free bilirubin concentration is thus hardly perceptible in the Sephadex method but is easily measured with the peroxidase technique

Woolley & Hunter (20) using an ultracentrifugation technique have likewise found that no displacement is observed with 4 mol fatty acid while 8 mol oleate causes complete liberation of bilirubin from albumin Circular dichroism studies however indicate that one mol oleate causes a perturbation of the primary binding site for bilirubin

The present results confirm that no displacement can be demonstrated by the first 4 molecules of fatty acid even with the sensitive peroxidase method Displacement of bilirubin (0 5 mol) from the primary binding site takes place however when more than 4 mol fatty acid is bound to albumin while the Sephadex method according to Starinsky & Shafrir indicates that larger amounts of fatty acid are needed

The binding of fatty acids to human plasma albumin has been studied by Goodman (8) and by Spector & Fletcher (18) These authors agree that about 6 or 7 molecules are bound with relatively high affinity and a large number to subordinate sites

As indicated by the present findings the first 4 sites for fatty acids do not bind bilirubin The 5th molecule of fatty acid is bound to the 1st bilirubin binding site The 2nd and 3rd site for bilirubin (2 9) are not influenced by fatty acid on the first 6 or 7 fatty acid sites

Addition of palmitate to normal adult sera causes no displacement of bilirubin even with 6 mol of the fatty acid per mol albumin This may be explained by binding of palmitate to other proteins or by a difference of the binding properties of the native albumin in adult plasma and the purified albumin preparation Sera from newborns behave in this respect as solutions of the pure albumin

In vivo in the human adult about one half site is normally occupied by fatty acid and usually from one to two sites in the newborn Concentrations of non-esterified fatty acid probably never exceed 4 mol per mol albumin and displacement of bilirubin by fatty acids thus seems excluded

SUMMARY

Displacement of bilirubin by fatty acids from the binding to human plasma albumin is studied by measuring increases of oxidation rate of bilirubin with peroxidase and ethyl hydroperoxide Amounts of fatty acid in excess of 4 mol per mol albumin are needed to cause displacement equally in the presence of 0 5 or 1 8 mol bilirubin Actual concentrations of non-esterified fatty acids *in vivo* even in the newborn are thus too low to cause any displacement of bilirubin from binding to albumin

Note added in proof Zamet & Chunga (This Journal 60 33 1971) have found a considrable displacement of bilirubin after infusion of fatty acids in an amount corresponding to 10 mol FFA per mol albumin in good agreement with the above *in vitro* findings

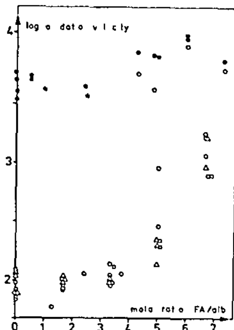


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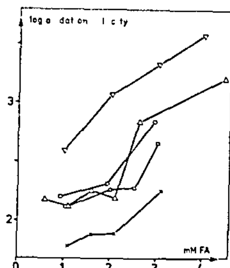


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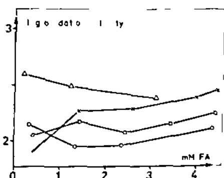


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HEALTH CONTROL OF FOUR YEAR-OLD CHILDREN

A Study of Bacteriuria

L. KOHLER H FRITZ and B SCHERSTÉN

From the Department of Paediatrics and Medical Microbiology University Hospital of Lund and Dalby Community Health Research Center Dalby Sweden

Urinary tract infections originating in child hood may be responsible for progressive renal disease detected first in adult life (10 18) Several studies have also shown that asymptomatic bacteriuria is often associated with symptomatic disease later on Kunin (7) showed that 10 3 of girls with asymptomatic bac teriuria had clinical episodes of acute pye lonephritis Smellie (19) found that children with uncontrolled asymptomatic infections sometimes proceeded to progressive scar for mation and renal contraction Accordingly, bacteriuria seems to merit detection and treat ment as early as possible in childhood (8) If such preventive measures are to be effective they should be included in the organized gene ral health service program for children Dur ing the first years of life however the obvious difficulties in obtaining adequately collected urine samples make diagnostic as well as screening methods unreliable

This report demonstrates the possibility of performing an investigation of bacteriuria on 4-year-old children as part of a general health control The reliability of the bacteriological techniques and the screening procedures are evaluated

MATERIAL

In an effort to bridge the gap of efficient health control of children belonging to age groups between infancy and school age a comprehensive study of all 4-year-old children was started in 1967 in the city of Lund and in the community of Dalby in the southern part of Sweden (5)

All children of 4 years of age living in these areas were selected from the county population register There was a total of 1 606 4-year-old children 1 459 living in Lund 1967 1969 and 147 in Dalby 1968

METHODS

The children were invited to participate by a letter to their parents The invitation was accompanied by questionnaires regarding *inter al a* previous symptoms and present complaints of the urinary tract (treated by physician for urinary tract infections previous symptoms of cystitis but not treated by physician present urgency frequency or dysuria) No effort was made to confirm the information from any records

The first morning urine was collected at home as a midstream sample without prior periurethral cleansing Fasting and thirsting during the night was required and the child should not have voided during the previous 4–6 hours The time for collection of the urine sample and that of the previous micturition was recorded by the parents The sample was chilled immediately with ice and brought to the child health centre the same day Details of the instructions have been described previously (2)

Laboratory procedures

The urine was examined for glucose semiquantitatively by Uriglox® (16) Absence of colour reaction indicates hypoglucosuria a sign of bacteriuria (2, 14 17)

Examination for proteinuria and hyperglucosuria was made by Labstix®

Quantitative bacteriological cultures were performed by the calibrated loop technique (1 2 3) The criteria for significant bacteriuria according to Kass (4) were used

ACKNOWLEDGEMENTS

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Table 3 *Bacteriological findings in the first collected urine specimens from 1238 4 year-old children*

Organism	Number of subjects with No growth	$<10^4$	10^4–10^5	$>10^5$	Total	*
No growth	886				886	71 6
E coli		25	24	9	58	4 7
Coliforms		3	12	5	20	1 6
Enterococci		43	34	1	78	6 3
Staphylococci[a]		11	9		20	1 6
Proteus mirabilis		22	21	3	46	3 7
Pseudomonas aeruginosa			3		3	0 2
Urinary nonpathogens[b]		113	13	1	127	0 3
Total number	886	217	116	19	1238	100 0
Percent	71 6	17.5	9 4	1.5	100 0	

[a] Coagulase negative
[b] Micrococci diphtheroids streptococci as a rule in a mixed flora

significant bacteriuria with enterococci was found

E coli were demonstrated in 54 girls and 4 boys In 9 subjects all girls these organisms were found in $>10^5$ these findings were reproducible from new samples in 4 girls only

Coliforms (*Enterobacter*) were isolated in 14 girls and 6 boys Of these 5 girls had $>10^5$ this amount being reproducible in 2 girls

Proteus mirabilis were found in the urine of 46 children 29 boys and 17 girls all non reproducible at a level of $>10^5$

Thus 6 girls had significant bacteriuria 4 with growth of *E coli* and 2 with growth of coliforms This gives a prevalence rate of 0 8% among the 711 girls No boy was found with significant bacteriuria

Table 4 gives the results of consecutive cultures performed from the 1 238 children Two consecutive samples with growth of >10 and with the same urinary pathogens (significant bacteriuria) gave a confidence level of 100% in predicting reproducible findings in two further samples

The frequency of contamination i e non reproducible findings of $>10^5$ of urinary pathogens within the five series of samples was found to vary between 1 1% and 2 1% (mean 1 2%) (Table 4) These rates of contamination did not differ significantly from each other ($p>0\ 05$) Thus the tendency to

Table 4 *Results of cultures from 2 009 samples in 1 238 4 year-old children*

Sample	No of samples	Number of subjects with No growth or $<10^5$	Growth of $>10^5$	Growth of $>10^5$ reproducible	Growth of $>10^5$ non reproducible	Percentage of contamination at a level of $>10^5$
First	1 238	1 219	19	6	13	1 1
Second	425	414	11	6	5	1.2
Third	200	191	9	6	3	1 5
Fourth	102	94	8	6	2	2 1
Fifth	44	44	0	—	0	0
Total	2 009	1 962	47	6	23	Mean frequency of contamination 1.2

No further samples were collected from these 6 subjects before treatment

Table 1 *Number and percentage of 4 year old children participating in the study of bacteriuria*

	Invited children			Investigated children					
				Boys		Girls		Total	
	Boys	Girls	Total	*n*		*n*		*n*	%
Living in Lund	747	712	1 459	681	91 2	644	90 5	1 325	90 8
Living in Dalby	70	77	147	64	91 4	67	87 0	131	89 1
Total	817	789	1 606	745	91 2	711	90 1	1 456	90 7

Follow up

In all subjects with bacterial colony counts representing 1 000 or more organisms per ml of urine new samples were collected Un coloured Uriglox® or proteinuria were always followed by collection of new samples Those who had not followed the instructions in all respects were requested to deliver new samples

Clinical Examination

All children passed a comprehensive pediatric examination the day of screening for bacteriuria Cases with significant bacteriuria or reproducible findings of 10 or more urinary pathogens per ml (37 children) were re examined at the Department of Paediatrics The examination included blood pressure white blood cell count hemoglobin concentration erythrocyte sedimentation rate serum urea N urinary microscopy and urinary osmolality Intravenous pyelography and micturition urethro-cystography were performed on selected cases

RESULTS

Participation in the study Out of the 1 459 children in Lund and 147 children in Dalby 1 325 (90 8%) and 131 (89 1%), respectively, participated in the study (Table 1)

Effectiveness of the instructions for collection of the urine specimens The number and percentage of children who followed the instructions in different respects are shown in Table 2 The important request not to void for at least 4–6 hours before delivering the sample was followed by all but 36 children (2 5%) *19 of these 36 children had nocturnal* enuresis In the remaining 17 children the instruction was not understood or the retention time not recorded The special instructions necessary for screening with the glucose method i e fasting and thirsting during the night, were followed by about 99% Thus the instructions necessary for the screening procedure were followed better than those necessary for bacteriological culture 7 1% did not chill their samples and 3 4% did not void directly into the test tube In total 1 238 out of the 1 456 children followed the instructions in every respect (85%)

Table 2 *Effectiveness of the instructions for collection of the urine specimens from 1456 4 year-old children*

	Number of children following instructions	
Fasting	1 440	98 9
Thirsting	1 445	99 2
Micturition directly into the test tube	1 406	96 6
Urine sample chilled	1 353	92 9
Incubation time in the bladder ≥6 hours	1 368	94 0
Incubation time in the bladder ≥4 hours	1 420	97 5

Bacteriological findings The results of the bacteriological cultures from the first collected urine specimens from the 1 238 children who followed the instructions in every respect are given in Table 3 No growth was found in 71 6% $<10^3$ or 10^3–10 in 26 9% and >10 *in 1 5% The frequency of samples with no* growth was the same in both sexes More than 10^5 organisms per ml was found in 19 subjects 5 boys and 14 girls

Enterococci were the most frequently isolated organisms being found in 78 children of whom 54 were boys However no case of

of urine A radiological study revealed vesico-ureteric reflux on both sides ureteral con struction and a dilated renal pelvis on the left side Seven urine samples had been col lected during the study four gave growth of *Proteus mirabilis* numbering <10³ and 10³–10⁵ and three gave no growth

DISCUSSION

The present study shows that it is possible to perform a screening program for bacteriuria at the age of 4 years This age was chosen because the children were expected to be able to cooperate To obtain the desirable high attendance in a screening program the parents must recognize the investigation as being of importance for their children s health The attendance of 90% in our study was attained probably because the screening for bacteriuria was included in an extensive general health control

In order to separate bacteriuria from conta mination by quantitation of urinary pathogens standardized procedures for the urine sampling are necessary (10) A remarkably high percent age of our preschool children fulfilled these demands 92 9% had their samples chilled and the imperative claim of at least 4–6 hours of retention of the urine in the bladder was followed by 97 5% In addition 99 2% did not drink and 98 9% did not eat during the night These results compare favourably with those obtained in schoolgirls and adults (2)

Kunin et al (6) in studying children and Norden & Kass (10) in studying adults have shown that the frequency of contamination at a level of >10⁵ with growth of urinary pathogens may be as high as that of significant bacteriuria in apparently healthy subjects Their conclusions were drawn from studies of midstream samples after thorough periurethral cleansing Savage et al (13) by collecting mid stream specimens at school without cleansing or spreading of the labia but under the super vision of a nurse found a contamination rate of 1 8% Our frequency of contamination at a level of >10⁵ was 1 2% This rather low contamination rate was obtained by written instructions to use midstream sampling at home without periurethral cleansing This sampling technique was chosen to simplify the collection of the first morning urine for the children and the parents

With our technique of collecting the urine at home the confidence level of *two* conse cutive samples in predicting reproducible find ings of >10⁵ organisms per ml of urine was sufficiently high for the diagnosis of bac teriuria (10) During the close follow up of the children with growth of 10⁵ or less organisms per ml in their initially collected samples no case of significant bacteriuria appeared Thus provided the urine is incubated in the bladder for at least 4–6 hours the level of >10⁵ organisms per ml of urine seems to be a true dividing line between bacteriuria and conta mination

The frequency of significant bacteriuria found in this unselected population of 4-year old children was 0 8% of the girls No case of significant bacteriuria was found among the boys The boy who later developed an overt urinary tract infection was found to have radiological abnormalities This case was not disclosed either by the screening method or by the bacteriological technique in his asymp tomatic phase

Few studies have been published regarding the prevalence of bacteriuria in apparently healthy children of the preschool age group

In their study of 5 year-old girls Savage et al (13) found a frequency of bacteriuria of 2 1% the survey comprised about 63% of the 5 year-old girls of the district

In a survey of 1 005 apparently healthy chil dren between 3 and 7 years old comprising 40% of the age group Righard (12) found significant bacteriuria in 0 9% of the girls and no case among the boys

Meadow et al (9) investigated 178 girls at the age of 5–7 years and diagnosed bacteriuria in 1 7%

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Table 5 *Evaluation of Uriglox® as a screening test for significant bacteriuria in 1456 4 year old children*

	No of subjects with significant bacteriuria	No of subjects without significant bacteriuria	Total
No colour (indication of bacteriuria)	6	12	18
Normal green colour	0	1 438	1 438
Total children	6	1 450	1 456

Evaluation sensitivity = 100 specificity = > 99

contaminate at a level of > 10 was random in this study There was no sex difference in the tendency to contaminate, $p > 0.05$

Uriglox For evaluation of the test paper Uriglox® in screening for bacteriuria the total material of 1 456 4 year old children was considered (Table 5) Absence of test paper colour reaction was considered to be an indication of bacteriuria and a colour reaction to be normal Out of the 1 456 children 6 had significant bacteriuria and of these the test paper disclosed all i e the sensitivity of the screening method was 100% Among the 1 450 children without significant bacteriuria the test paper gave a colour reaction for 1 438 (> 99%) and thus a false indication of bacteriuria in less than 1%

Past histories Past history of earlier urinar tract infections was common 5 8% (54 girl and 30 boys) as was present complaints o cystitis', 5 9% (61 girls and 25 boys) Onl very few of these children however ha significant bacteriuria (Table 6)

Proteinuria haematuria and hyperglucosuria Proteinuria, as determined by Labstix® was present in 14 children 1 0% (Table 6) N child had growth of $> 10^5$, and the proteinuri was not persistent in further samples

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Clinical re examination A re examinatio with special regard to the urinary tract wa performed on 37 children The osmolality o the first morning urine varied between 714 and 1 336 mosm/kg and in the cases with signifi cant bacteriuria between 805 and 1 028 mosm/kg Leucocyturia (> 10 cells/mm³) was found only in one case, a girl with bacteriuria Urea N were all within the normal range (< 20 mg%) Intravenous pyelography and micturi tion cystography were made in 4 girls with significant bacteriuria and in 2 boys with re producible growth of $\leq 10^5$ of *Proteus mirabilis* No abnormal findings were recorded The remaining 2 girls with significant bacteriuria refused X ray examinations

Nine months after the initial urine ex amination one boy fell ill with a high fever dysuria and pyuria Bacteriological culture gave growth of $> 10^5$ *Proteus mirabilis* per ml

Table 6 *Past history and present symptoms from the urinary tract in 1456 4 year old children*

	Results of cultures					
Symptoms	No growth	Growth of $< 10^3$ or 10^4–10^5	Growth of $> 10^5$	Significant bacteriuria	Total	
Treated by physician for urinary tract infection	59	21	3	1	84	5 8
Previous symptoms of cystitis but not treated by physician	19	7	1	0	27	1 9
Present complaints of urgency frequency or dysuria	61	22	0	3	86	5 9
Present proteinuria (Labstix®)	11	3	0	0	14	1 0
Present hyperglucosuria (Labstix®)	0	0	0	0	0	0 0

of urine A radiological study revealed vesico-ureteric reflux on both sides ureteral con struction and a dilated renal pelvis on the left side Seven urine samples had been col lected during the study four gave growth of *Proteus mirabilis* numbering $<10^3$ and 10^3–10^4 and three gave no growth

DISCUSSION

The present study shows that it is possible to perform a screening program for bacteriuria at the age of 4 years This age was chosen because the children were expected to be able to cooperate To obtain the desirable high attendance in a screening program the parents must recognize the investigation as being of importance for their children s health The attendance of 90% in our study was attained probably because the screening for bacteriuria was included in an extensive general health control

In order to separate bacteriuria from conta mination by quantitation of urinary pathogens standardized procedures for the urine sampling are necessary (10) A remarkably high percent age of our preschool children fulfilled these demands 92 9% had their samples chilled and the imperative claim of at least 4–6 hours of retention of the urine in the bladder was followed by 97 5 In addition 99 2 did not drink and 98 9 did not eat during the night These results compare favourably with those obtained in schoolgirls and adults (2)

Kunin et al (6) in studying children and Norden & Kass (10) in studying adults have shown that the frequency of contamination at a level of $>10^5$ with growth of urinary pathogens may be as high as that of significant bacteriuria in apparently healthy subjects Their conclusions were drawn from studies of midstream samples after thorough periurethral cleansing Savage et al (13) by collecting mid stream specimens at school without cleansing or spreading of the labia but under the super vision of a nurse found a contamination rate of 1 8 % Our frequency of contamination at a level of $>10^5$ was 1 2% This rather low contamination rate was obtained by written instructions to use midstream sampling at home without periurethral cleansing This sampling technique was chosen to simplify the collection of the first morning urine for the children and the parents

With our technique of collecting the urine at home the confidence level of *two* conse cutive samples in predicting reproducible find ings of $>10^5$ *organisms per ml of urine* was sufficiently high for the diagnosis of bac teriuria (10) During the close follow up of the children with growth of 10^5 or less organisms per ml in their initially collected samples no case of significant bacteriuria appeared Thus provided the urine is incubated in the bladder for at least 4–6 hours the level of $>10^5$ organisms per ml of urine seems to be a true dividing line between bacteriuria and conta mination

The frequency of significant bacteriuria found in this unselected population of 4 year old children was 0 8% of the girls No case of significant bacteriuria was found among the boys The boy who later developed an overt urinary tract infection was found to have radiological abnormalities This case was not disclosed either by the screening method or by the bacteriological technique in his asymp tomatic phase

Few studies have been published regarding the prevalence of bacteriuria in apparently healthy children of the preschool age group

In their study of 5 year-old girls Savage et al (13) found a frequency of bacteriuria of 2 1% the survey comprised about 63% of the 5 year old girls of the district

In a survey of 1 005 apparently healthy chil dren between 3 and 7 years old comprising 40% of the age group Righard (12) found significant bacteriuria in 0 9% of the girls and no case among the boys

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4-year-old children Preliminary results of a pilot study *Acta Paediat Scand 58* 639 1969
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pointed out that bacteriuria was twice as prevalent (1 4%) among the younger girls (aged 6–7 years) than among slightly older girls (aged 8–9 years)

Our results show that the prevalence of bacteriuria is about the same at the age of 4 years (0 8%) as among schoolgirls aged 7–18 years (1 5%) ($p > 0\ 05$) in the same city (15) The prevalence of bacteriuria in 4 year old girls seems high enough to motivate screening which, however should be repeated at school The well known extremely low rate of bacteriuria among boys above infancy was confirmed in this study and thus, screening is not motivated in preschool boys

The questionnaire regarding previous symptoms from the urinary tract did not select a risk group of children with a high frequency of bacteriuria nor did the examination for proteinuria (Table 6) Out of the 86 children with present complaints only 3 girls had significant bacteriuria These findings support an assumption that the very high frequency of children earlier treated for urinary tract infection, 5 8% is reached by over diagnosis (11) Among the 6 children with significant bacteriuria 3 had actual symptoms (Table 6) This gives a sensitivity of 50% and a specificity of 94 3% if the questionnaire regarding actual symptoms is used for screening of significant bacteriuria Although the questionnaires revealed present complaints among half of the patients with bacteriuria, these symptoms had not brought the children to a doctor

The screening results obtained by the glucose method as performed semiquantitatively by Uriglox® show a very good agreement with the bacteriological cultures Hypoglucosuria gives suspicions of multiplication of micro organisms in the urinary tract above the urethra to the same extent as *one* quantitative urinary culture with findings of $> 10^5$ organisms per ml The combined results of hypoglucosuria (no colour of Uriglox®) in one sample and growth of more than 100 000 urinary pathogens in a second sample is diagnostic of bacteriuria The glucose method as performed with Uriglox® offers an opportunity of screening for bacteriuria with a high degree of sensitivity and specificity in 4-year old children

SUMMARY

In the present study 1 456 four year-old children were investigated for bacteriuria as a part of a general health control The attendance rate was 90% The urine samples were collected at home under standardized conditions Screening for bacteriuria was performed with Uriglox®, quantitative bacteriological cultures were made in all children

The results showed that the urine sampling at home was adequate both for bacteriological and for screening purposes The prevalence of significant bacteriuria among the girls was 0 8% No boy had significant bacteriuria The screening method disclosed all the bacteriurics and more than 99% of the non bacteriurics Neither the questionnaire regarding past and present symptoms of the urinary tract nor the finding of proteinuria were suitable as screening instruments for bacteriuria

Findings of 10^5 or less organisms per ml were insignificant The frequency of contamination was 1 2% A positive screening result with Uriglox® followed by the demonstration of $> 10^5$ of urinary pathogens per ml in *one* sample was diagnostic of bacteriuria

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CORRECTION OF THE DEFECTIVE SULFATIDE DEGRADATION IN CULTURED FIBROBLASTS FROM PATIENTS WITH METACHROMATIC LEUCODYSTROPHY[1]

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A number of single hereditary lysosomal enzyme deficiencies result in severely disabling metabolic disorders for which no current therapy is known Aside from the correction of the defective gene by bioengineering the replacement of the missing lysosomal enzyme could prove to be an effective method in the treatment of single lysosomal enzyme deficiencies

Metachromatic leucodystrophy (MLD) a disease with a known enzyme deficiency which results in a well established substrate degradation defect, provided us with the opportunity to investigate at the cellular level the effect of enzyme substitution upon the metabolic disorder

Late infantile MLD is a genetic disorder characterized by progressive demyelination and mental retardation Large amounts of cerebroside sulfate (sulfatide) accumulate in the nervous system and in some visceral organs The underlying defect is the absence of a lysosomal enzyme arylsulfatase A (galacto cerebroside sulfatase) which degrades sulfatide into cerebroside and sulfate (1 2 11) This enzyme is also missing in cultured fibroblasts of the patients However no accumulation of sulfatide takes place, since it is not synthesized in these cells Exogenously administered ^{35}S sulfatide is taken up by normal fibroblasts and broken down into cerebroside and $^{35}SO_4$ In the patient s cells this process is impaired (13)

We have found that arylsulfatase A concentrated from normal urine and added to the culture medium is taken up by the fibroblasts In MLD fibroblasts the substituted enzyme showed characteristics of the urinary arylsulfatase A and remained active for more than 9 days The enzyme replacement resulted in an increased degradation of the intracellular ^{35}S sulfatide to dialysable ^{35}S This occurred if the ^{35}S sulfatide was given simultaneously with the enzyme preparation as well as if the cells had been labeled with ^{35}S sulfatide before or after the enzyme substitution

MATERIAL AND METHODS

Fibroblast cultures from 3 patients with the clinical diagnosis of late infantile metachromatic leucodystrophy were used in this study The clinical and the laboratory findings in the patients are summarized in Table 1 Fibroblasts from skin biopsies of 6 normal children of a comparable age group served as controls

The fibroblasts were grown from skin biopsies obtained under local anesthesia by punch biopsies during minor surgical operations Sp cial care was taken not to use disinfectants which contain mercury which was found to inhibit cell growth Primary cultures were grown in 50 ml plastic bottles (Falcon Plastic Becton Dickinson Co Baltimore Md) in Hams F 10 culture medium containing 2 fetal calf serum and 18 horse serum The established cell lines were maintained in 250 ml Falcon flasks in Eagle minimal

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Table 1 *Clinical and laboratory findings in the patients investigated*

Patients	Sex	Age at onset (months)	Age at biopsy (months)	Clinical stage (7) at biopsy	Arylsulfatase A (Urine) (U/ml)	Sulfatide excretion (24 hr urine)	Nerve conductivity decreased	Nerve biopsy metachromesia
MLD-1	F	20	24	II	<1 U/ml	170 µg	+	
MLD-2	F	14	~27	III	<1 U/ml	70 µg	+	+
MLD-3	M	18	30	III	<1 U/ml	400 µg	+	+
Normal values					>5 U/ml	0–11 µg	−	−

essential medium and Earle salt supplemented with additional 300 mg glutamine and 10% fetal calf serum The cultures were fed twice weekly and subcultured at intervals of 2 to 4 weeks. All cultures were incubated at 37 C in an atmosphere of 95% air and 5% CO_2. The pH of the culture media were 7.4 At the time of the experiments all the cells had undergone about the same number of generations and were used when they were grown to confluency Each bottle contained from 2–4 mg of cell protein and between 3–6 × 10^6 cells The cells were harvested by trypsinisation with 0.5% trypsin centrifuged and washed once in 0.9% saline After centrifugation the cells were either extracted with 12 ml chloroform-methanol 1 1 for lipid estimations or sonicated in 2 ml of 0.9% saline twice for 30 sec in a Branson Sonicator (Branson Sonic Power Co Danbury Conn)

Arylsulfatase A activity was determined in the sonicated cells according to Austin et al (1) The enzyme activity was expressed as 1 U = 1 nanomole substrate cleaved per hr Arylsulfatase A activity was concentrated from normal urine by precipitation with ammonium sulfate (80% of saturation) at 0°C The precipitate was centrifuged at 20 000 g for 30 min and reconstituted to 1 50 of the original volume with distilled water After exhaustive dialysis against distilled water and finally against saline the arylsulfatase A activity was found to be 1 500 U per ml concentrate The recovery of the arylsulfatase A activity was over 80% The pH optimum of the arylsulfatase A activity in this preparation was 5.0

^{35}S-sulfatide was prepared by injecting carrier free $^{35}S\text{-}Na_2SO_4$ in saline into the peritoneum of 16-day-old rats every other day for 1 week The lipids were extracted from the brain by the method of Folch (4) except that—instead of one partitioning with theoretical upper phase—five washes were used The lipids were separated by two-dimensional thin layer chromatography (TLC) and ^{35}S-sulfatide isolated and estimated (8) The specific activity was 350 µCi/mmole ^{35}S-sulfatide was solubilized by saponification in 0.5 N NaOH and neutralized with 0.5 N HCl 50 000 dpm of ^{35}S-sulfatide were added to each of the 250 ml culture bottles each of which contained 15 ml of medium The physical and chemical characteristics of ^{35}S-sulfatide in the medium were proven to be unchanged by this procedure The ^{35}S-sulfatide could be extracted quantitatively from the medium without loss of radioactivity No dialysable ^{35}S could be demonstrated ^{35}S-sulfatide was extracted from the trypsinized and washed cells and from a 2 ml aliquot of the medium as described above An aliquot of the medium was dialysed against 0 1 M ammonium sulfate followed by an exhaustive dialysis against distilled water

In order to calculate non-lipid ^{35}S aliquot from cells or medium were taken and total ^{35}S was measured Then the lipid soluble ^{35}S was extracted and estimated Recovery studies showed that the total ^{35}S was distributed only as ^{35}S-sulfatide and water-soluble dialysable ^{35}S No ^{35}S was found in the chloroform methanol insoluble residuum

The amount of dialysable ^{35}S was equal to the difference between the total ^{35}S minus ^{35}S-sulfatide Radioactivity was measured by liquid scintillation in a Tricarb counter (Packard Instrument Co Downers Grove Ill) equipped with an absolute activity analyzer The results are expressed as disintegrations per minute (dpm)

EXPERIMENTAL

Enzyme replacement in fibroblasts

Cell cultures from the patients and from normals were maintained in a medium to which arylsulfatase A preparation was added (40–80 U/ml medium) Intracellular arylsulfatase A activity was determined in control cultures which had not received enzyme preparations and in test cultures after the 3-day incubation in the arylsulfatase A-containing medium Then the medium was removed and fresh medium was added at intervals of 3 days Arylsulfatase A activity was determined in the cells at day 3 6 and 9

Correction of the faulty sulfatide metabolism

In order to study the effect of the enzyme replacement on the defective degradation of ^{35}S-sulfatide in the fibroblasts three different types of experiment were performed

1 Fibroblast cultures from normals and from MLD patients were labeled with 50 000 dpm ^{35}S-sulfatide added to 15 ml of medium in each culture flask At the same time arylsulfatase A preparation was adde to test cultures of each cell line until the activit of arylsulfatase A was 60 U/ml of medium An al quot of saline was added to the medium of the contr cultures After a 3 day incubation ^{35}S-sulfatide an

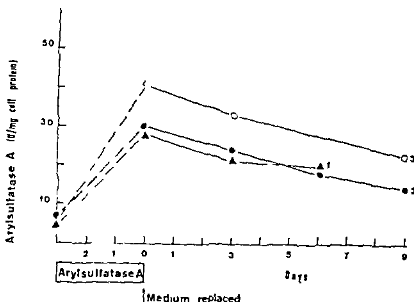


Fig 1 Enzyme replacement in fibroblasts Decrease of the substituted aryl sulfatase A activity in MLD cells ▲ MLD 1 ● MLD 2 ○ MLD 3

arylsulfatase A activity were determined in the cells Dialysable ^{35}S total ^{35}S and ^{35}S-sulfatide in the medium were measured in all cultures

2 Identical cultures grown from normal and from MLD cell lines were prelabeled for 3 days in a medium containing 35 000 dpm ^{35}S-sulfatide in 15 ml medium per culture At the end of this period the medium was removed and arylsulfatase A activity and ^{35}S-sulfatide were determined in one culture The cells of the other cultures were washed *in situ* with sterile saline and incubated for a second period of 3 days in sulfatide free medium An arylsulfatase A preparation was added to the medium of the test cultures An aliquot amount of saline was given to the control cultures Arylsulfatase A activity in the medium was about 50 U/ml At the end of this period the enzyme activity and ^{35}S-sulfatide content were measured in the cells The total dialysable ^{35}S and ^{35}S-sulfatide were determined in the medium

3 Cultures from cell lines from MLD patients were loaded with arylsulfatase A (50 U/ml of medium) for 3 days then the medium was removed and replaced by fresh medium Beginning on the third sixth and ninth days thereafter ^{35}S-sulfatide (50 000 dpm per culture flask) was added for three consecutive days The cells were then harvested and arylsulfatase A activity and ^{35}S sulfatide content were measured in the cells The total dialysable ^{35}S and ^{35}S sulfatide were determined in the medium

Control experiments

Arylsulfatase A preparation and ^{35}S-sulfatide were incubated at 37°C pH 7 4 for 3 days in a medium without cells in order to study the possible breakdown of ^{35}S-sulfatide to dialysable ^{35}S at a pH which was identical to the one at which the cells were cultivated during the experiments

RESULTS

The addition of arylsulfatase A preparation to the culture medium over a period of 3 days resulted in an increase in the intracellular activity of this enzyme in the fibroblasts In cells from the patients the activity rose from an original 2–5 % up to 15–25 % of the normal values In normal cells treated with enzyme preparation up to 30 % higher values were

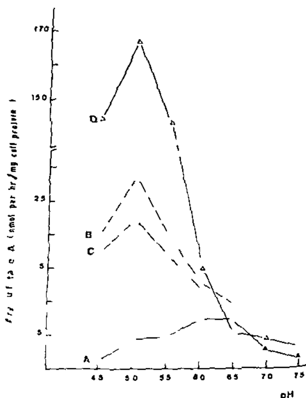


Fig 2 pH optimum of the arylsulfatase A activity in normal fibroblasts and in MLD cells before and after enzyme substitution A MLD cells B MLD cells 3 days after enzyme substitution C MLD cells 6 days after enzyme substitution D Normal cells

Table 2 *Accumulation of intracellular ^{35}S sulfatide in the presence of urinary arylsulfatase A activity in the medium*

Patients	Control cultures			Test cultures		
	Intracellular arylsulfatase A[a]	^{35}S-sulfatide accumulated	^{35}S-sulfatide degraded[b]	Intracellular arylsulfatase A	^{35}S-sulfatide accumulated	^{35}S-sulfatide degraded[b]
1 MLD-1	5 0	3 400	290	51.5	1 640	2 600
2 MLD-	8 0	4 200	500	76 0	2 050	2 300
3 MLD-3	7 6	3 700	290	35 6	1 560	2 070
4 Normal	200 0	2 700	6 300	172.0	1 650	3 300
5 Normal	2-8 0	2 000	5 100	220 0	1 280	3 700

Arylsulfatase A activity U/mg cell protein
[b] *Measured as dialysible* ^{35}S *released into the medium (dpm/mg cell protein)*
dpm ^{35}S-sulfatide mg cell protein

found In the patient s cells the substituted enzyme remained active for more than 9 days although its activity diminished slowly during this time (Fig 1) The residual arylsulfatase A activity in untreated cells was between 5 and 9 U/mg cell protein measured at pH 5 0 Higher values were found when the enzyme activity was determined at pH 6 0 indicating a shift of the pH optimum in MLD cells (Fig 2) Since the activity of arylsulfatase B was found normal in MLD cells having the pH optimum at 6 0 (14) it was thought that arylsulfatase B was accounting partially for the residual A activity In MLD cells treated with arylsulfatase A preparation the pH optimum was found at pH 5 0 just as in normal cells indicating an increase in intracellular arylsulfatase A activity This shifted pH optimum remained constant as long as an increased enzyme activity could be measured in the cells

The results of the simultaneous administration of arylsulfatase A preparation and ^{35}S sulfatide are shown in Table 2

Compared with the untreated MLD cultures a decrease in the ^{35}S-sulfatide accumulated in the cells and a marked increase of the dialysable ^{35}S in the medium over the untreated control cultures was noted in all MLD cell lines receiving enzyme preparation An increase in the activity of intracellular arylsulfatase A was also observed The addition of a boiled enzyme preparation failed to show either an increased ^{35}S-sulfatide degradation to dialysable ^{35}S or a rise in enzyme activity Control experiments in which urinary arylsulfatase A preparation and ^{35}S-sulfatide were incubated at 37 C pH 7 4 for 3 days in medium without cells did not show a breakdown of ^{35}S-sulfatide to dialysable ^{35}S

Correction of the defective ^{35}S-sulfatide degradation could also be demonstrated in MLD cells by the addition of arylsulfatase A preparation to the medium of cells that had previously accumulated ^{35}S-sulfatide The results from these experiments are summarized in Table 3 The MLD fibroblasts accumulated about twice as much ^{35}S-sulfatide over the 3 day period as did the normal cells The addition of arylsulfatase A preparation to the medium of ^{35}S-sulfatide labeled cells again increased the intracellular activity of this enzyme particularly in the MLD fibroblasts At the same time the degradation of ^{35}S-sulfatide measured as dialysable and not lipid soluble ^{35}S in the medium was increased eight times over the untreated controls Expressed as a percentage of the intracellular ^{35}S sulfatide accumulated the degradation was completely normalized in the MLD cells which contained the substituted enzyme No effect on the degradation rate of the intracellular ^{35}S-sulfatide was observed in identically treated normal cells

The duration of the correction of ^{35}S-sulfatide metabolism by enzyme replacement was studied in MLD cells which were treated with

Table 3 *Correction of ^{35}S sulfatide degradation*
Period I ^{35}S sulfatide accumulation *Period II* ^{35}S sulfatide degradation

			Control			Test		
	Intra cellular arylsulfa tase A[a]	^{35}S sulfa tide accu mulated[d]	Intra cellular arylsulfa tase A[a]	^{35}S sulfa tide de graded[b]	Degra dation[c]	Arylsulfa tase A[a]	^{35}S sulfa tide de graded[b]	Degra dation[c]
Normal	138 (104–168)	793 (710–950)	143 (90–190)	510 (430–630)	64 3	222 (136–309)	425 (350–500)	53 6
Metachro matic leuco dystrophy	5 0 (4 0–6 0)	1 740 (1 200–2 440)	3 7 (2 9–4 4)	150 (0–230)	8 6	37 (26 5–40 0)	1 160 (780–1 500)	66 6

[a] Arylsulfatase A activity U/mg cell protein
[b] Measured as dialysable ^{35}S released into the medium (dpm/mg cell protein)
[c] ^{35}S sulfatide degraded in of ^{35}S sulfatide accumulated
[d] dpm ^{35}S sulfatide/mg cell protein

enzyme preparation previously to the labeling with ^{35}S sulfatide at different time intervals after the enzyme substitution had been com pleted A *significant correction of the* ^{35}S sulfatide metabolism could be demonstrated after 9 days in enzyme substituted cells as com pared with the untreated controls ^{35}S sulfatide accumulation *still remained 36%* lower *and* the amount of dialysable ^{35}S released into the medium three times higher than in the identi cal but untreated cultures

DISCUSSION

Fibroblasts in culture are capable of absorbing proteins and other macromolecules by pino cytosis from the surrounding medium (3) into the cells We assume that both arylsulfatase A and ^{35}S sulfatide accumulate in the cells by means of *such mechanisms The intracellular* location of the substituted arylsulfatase A activity can be substantiated by two observa tions

1 In the media containing the arylsulfatase A preparation the activity of arylsulfatase A at the end of the incubation period was always considerably lower than at the beginning and much below the enzyme levels in the cells

2 *In* enzyme substituted cells growing in medium without enzyme preparation the activ ity of intracellular arylsulfatase A disappeared only very slowly compared with a very steep gradient between cells and medium as is shown in Fig 1

The amount of ^{35}S sulfatide accumulated in the fibroblasts is the net result of the ^{35}S sulfatide taken up into the cells by pinocytosis *minus the degraded* ^{35}S sulfatide Since ^{35}S sulfatide can also leave the cells undegraded by a mechanism analogous to pinocytosis its accumulation is not necessarily a reliable parameter for the ^{35}S sulfatide metabolism Therefore *the correction experiments* were al ways expressed *on the basis of dialysable* ^{35}S in the medium rather than on the intracellular ^{35}S sulfatide

The mechanism by which a correction of a lysosomal enzyme deficiency occurs in cultured fibroblasts could be as follows The pino *cytosed macromolecules are stored in vacuoles* which are transformed into secondary lyso somes after fusion with the primary lysosomes containing the hydrolytic enzymes (5) Pro teins like albumin are rapidly degraded by the action of the acid hydrolases (15) lysosomal enzymes however seem to be particularly *resistant to inactivation even if they are* brought into the cells by pinocytosis rather than by being synthesized in the cells them selves Since ^{35}S sulfatide was degraded by the

substituted arylsulfatase A in the MLD cells it can be assumed that this degradation takes place in the lysosomes containing both the substituted enzyme and the substrate under conditions favorable for the activity of the enzyme These conditions are best met by the simultaneous uptake of arylsulfatase A and ³⁵S-sulfatide To explain the correction achieved in the experiments in which enzyme and substrate had been taken up at different times we have to assume the fusion of two secondary lysosomes one containing the enzyme the other the substrate in order that the degradation can take place

Fusion of secondary lysosomes was shown by Gordon et al for strain L fibroblasts (5)

A similar correction mechanism could be suggested for the experiments reported by Neufeld et al (12) where it was shown that normal urinary protein concentrates were able to correct the abnormal degradation of mucopolysaccharides in fibroblasts from Hurler and Hunter patients These diseases are also considered to be lysosomal enzyme deficiencies although no specific enzyme defect is known

Our model of a therapy by enzyme replacement in hereditary lysosomal enzyme deficiencies is of course not readily applicable to the patients themselves since—among other problems—a successful correction is limited to cells capable of active pinocytosis However it might be a step on the way to such possibilities

A few attempts have been reported in this direction none of which has resulted in an intracellular restoration of the defective metabolism (6 9 10)

SUMMARY

Late infantile metchromatic leucodystrophy is a hereditary disorder caused by a deficiency in the activity of cerebroside sulfatase (arylsulfatase A) In cultured fibroblasts from patients this enzyme is also deficient and ³⁵S-sulfatide degradation is impaired The enzyme activity can be partially replaced in tissue culture conditions by growing the cells in a medium containing arylsulfatase A There arylsulfatase A is taken up by the cells presumably by pinocytosis Concomitantly a correction of the degradation defect can be observed

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TIME OF THE FIRST URINATIONS IN MALE AND FEMALE NEWBORNS

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In contrast to the situation in older children and adults the occurrence of urinary tract infections during the neonatal period is much more common in male than in female newborns (2 3 5) On the basis of very reliable studies one can conclude that in neonatal urinary tract infections the male sex dominates with at least sevenfold frequency as compared with the females Since such an extremely clear-cut sex distribution is not found in any other neonatal infection—though the male sex may otherwise dominate slightly—one naturally tries to find a possible mechanical or functional disturbance in the genito-urinary tract of male newborns which would predispose to urinary tract infections A long stay of urine in the bladder and excessively high voiding pressure due to obstruction or neurogenic lesion are common causes of urinary tract infections Children who have no abnormalities of the urinary tract but who are infrequent voiders also seem to have an increased rate of urinary tract infections (1) Overdistention of the bladder has been supposed to damage the bladder wall and thus predispose to infection (1) Urinary tract obstruction of short duration seems to affect the resistance even more significantly than that of a longer duration (4) The long male urethra may be vulnerable during the birth process Urethral lesion and/or the "physiological phimosis" of a newborn boy might cause infrequent voiding and overdistension of the bladder Considering such a possible background for the urinary tract infections of male newborns we decided to study the time of the first and second voiding in male and female newborns The study would also give normal values of the time schedule of the first urinations

This work was assisted by a grant from The National Research Council for Medical Sciences Finland

SERIES OF PATIENTS

The first and second urination was recorded in 319 newborns born successively The material consisted of 155 boys and 164 girls All patients were physically examined by one of us (A L P) and no overt abnormalities, significant for this study were found Feeding schedule was the same for all the infants belonging to the study First feeding with 10% glucose was given when the babies were 6 hours old and subsequently they were fed *ad libitum* every 4th hour

RESULTS AND DISCUSSION

The percentage distribution of the time of passage of the first and second urine in female and male newborns is seen in Tables 1 and 2 The most significant difference between boys and girls was a much more frequent occurrence of the first urination at birth of the former (25% contra 7%) The first urination did not occur during the first 30 hours after birth in 2 6% of boys and in 1 2% of girls

Table 1 *Time of the first urination as percentages of total material*

	No	At birth	<5 h	5–10 h	11–20 h	21–30 h	>30 h
Boys	155	25	8	28	30	6	3
Girls	164	7	13	25	42	12	1
Total	319	16	11	26	36	9	2

No marked differences were noticed in the time of the second urination between females and males The infants with the first urination at birth voided the second time in the course of 5 to 45 hours There were 4 boys and 2 girls in the group with the first urination after 30 hours The first and second voiding both occurred in these 6 infants during the next 24 hours of life

In general, our results are in accordance with those of Sherry & Kramer (6) who found, in a series of 500 newborns of both sexes, that the first urination took place in the delivery room in 17%, during the first 12 hours in 50 6%, during 12–24 hours in 24 8% during 24–48 hours in 7% and thereafter in 0 6% In our series the average percentage of infants with the first urination at birth was 16 with a marked preponderance for male infants

The high frequency of male infants with the first voiding at birth is hardly pathogenetically associable with the frequent infections of the urinary tract In our material a delayed first voiding (after 30 hours) only occurred in 4 boys and 2 girls and a larger series is needed for confirmation of a male prevalence

Table 2 *Time of the second urination as percentages of total material*

	No	<10 h	11–20 h	21–30 h	>30 h
Boys	148	14	45	28	13
Girls	159	9	40	38	13
Total	307[a]	12	42	33	13

[a] In 12 of the original 319 infants data on the second urination were not available

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IMPAIRED CALCIUM HOMEOSTASIS IN THE INFANTILE HYPERCALCEMIC SYNDROME

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The etiology of the severe form of the infantile hypercalcemic syndrome (a rare condition which includes supravalvular aortic stenosis mental retardation and an "elfin facies") is not known nor has the origin of the hypercalcemia been established Current hypotheses include vitamin D hypersensitivity or excess an abnormality of cholesterol metabolism and delayed turnover or degradation of vitamin D Although a review of the literature led Seelig (28) to conclude that these patients have an increased response to vitamin D Fraser et al (14) are of the opinion that none of these hypotheses have been proven The hypercalcemia usually disappears during childhood but the other features remain

Although large oral doses of vitamin D will intensify the hypercalcemia in infants with this syndrome (28) modest doses do not alter serum calcium in older children (5 13 33) Barr & Forfar (6) and Dormandy & Begum (11) have reported that an oral calcium load resulted in higher and more sustained levels of serum calcium in infants with the syndrome than in controls older children on the other hand behaved normally in this respect an observation which we have confirmed (13)

During the investigation of a group of children with the idiopathic hypercalcemic syndrome we decided to study their response to an intravenous infusion of calcium In view of the known influence of hypothyroidism on the ability of the body to handle intravenous calcium loads (3 32) children with this disorder were included in the protocol

SUBJECTS AND METHODS[1]

There were eight children aged 4 to 15 years with the typical clinical features of the idiopathic hypercalcemic syndrome including elfin facies mental retardation and cardiovascular anomalies Six subjects had been referred for evaluation from the cardiac clinic and documentation of earlier blood calcium levels was not available Two subjects had been followed since early infancy with documented hypercalcemia requiring therapy with cortisone and a low calcium—low vitamin D diet both had increased bone density in infancy and in one the osteosclerosis was pronounced At the time of study all of the subjects had normal levels of serum calcium The cardiac lesions in these patients were consistent with those described by others and included supravalvular aortic stenosis, branch pulmonary artery stenosis coarctation of the aorta, and congenital mitral insufficiency Appropriate cardiac studies to document the cardiac anomalies had been performed in each subject All were retarded in mental development and exhibited the volatile talkative personality and the facial configuration so characteristic of this condition

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[1] Prior to inclusion in this study the subjects and their parents were informed of the experimental nature of this work the procedures to be followed and the potential hazards Only those who completely understood and accepted these studies were included

Table 1 *Intravenous calcium infusion (10 mg/kg)*

Subjects	n	Average serum Ca (mg/100 ml)		$t_{\frac{1}{2}}$ (min)		Urine Ca (averages)	
		Initial	Increment	Average	Range	Baseline (mg/hr)	Dose excreted in 3 hours
Control	6	9 8±0 7[1]	3 3±1 1	68[a]±24	40–108	5 0	8 0
Hypercalcemic	7	9 7±0 5	2 9±0 6	114[b]±42	37–162	3 5 (6)[2]	9 6
Hypothyroid	4	9 0±0 3	2 4±0 6	201[c]±78	112–281	1 1 (2)[2]	1 7

[1] standard deviation
[2] n
[a] vs [b] $p<0\,05$
[b] vs [c] $p<0\,05$

The control subjects were six children aged 4 to 15 years One was the normal sibling of a patient one had chronic moniliasis one was tall for age and three had short stature (at or slightly below the third percentile) of unknown cause None had clinical or laboratory evidence of pituitary thyroid parathyroid adrenal or gonadal disea e nor did skeletal roentgenograms show abnormalities in bone density or configuration

Also included in the study were four subjects aged 4 months to 10 years with untreated hypothyroidism Two were athyreotic cretins one had a thyroidectomy and one had juvenile myxedema unresponsive to TSH

The intravenous calcium infusions were given in the morning after an overnight fast A 2 hour sample of urine was collected and two blood samples obtained for calcium analysis Calcium gluconate diluted in 5 dextrose solution was then infused intravenously at a constant rate during the next hour in a dose of 10 mg Ca/kg body weight Blood samples were obtained at the end of the infusion and at 15 30 45 60 90 and 120 min post infusion Urine collections were continued during the infusion and throughout the period of sample collection No food was offered during this study

The increments in serum calcium concentration relative to the average of the two pre infusion samples were plotted on semi logarithmic paper and the time required for this increment to fall to one half of its maximum value was determined by least squares regression analysis Although the rate of post infusion fall of serum calcium undoubtedly is a more complex process than such a mathematical treatment assumes it to be a single exponential function appears to characterize the data reasonably well There was only one subject in whom the scatter of the data points precluded the construction of a satisfactory time plot

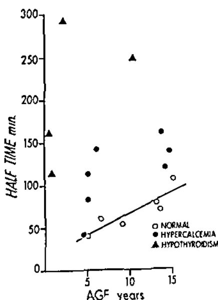


Fig 1 Calculated half times for the fall of incremental serum Ca plotted against age of subject Normal (O) hypercalcemic (●) documented hypercalcemia in infancy (●*) hypothyroid (▲) Regression line for normal subjects calculated by method of least squares

RESULTS

Table 1 summarizes the data The calculated half times for the incremental fall in serum calcium tend to be longer (indicating a slower rate of return to the pre infusion value) in the hypercalcemic subjects than in the controls The average half time in these subjects is 1 7 times that of the controls ($p<0\,05$) while that of the hypothyroid subjects is 3 0 times

The individual half time values are plotted according to age of the subjects in Fig 1 Here it can be seen that age has a decided effect on the half time in the controls The calculated regression line is $t_{\frac{1}{2}} = 5\,15A + 15\,8$ ($t_{\frac{1}{2}}$ in minutes A is age in years) [1] The cor

[1] Similar doses of intravenous calcium in adults yield calculated half times of 150–180 min (19 21) the equation predicts half times of these magnitudes by age 26–32 years

relation coefficient is 0 89 ($p<0\,05$) and the standard error of the slope is 1 325 indicating that the regression slope differs significantly from zero The derived equation for the "hypercalcemic" subjects is $t_{\frac{1}{2}} = 5\,76A + 62\,3$ (r 0 65) but the standard error of the regres sion slope (3 02) is such that the slope does not differ significantly from zero

This type of plot emphasizes the differences in half times among the three groups of subjects It also suggests that the distinction be tween the "hypercalcemic and control subjects in dealing with such a calcium load is greatest in the young child and that this dis tinction tends to lessen with age

DISCUSSION

It would appear that the impaired ability of the *infant* with the hypercalcemic syndrome to handle large calcium loads (6 11) persists into the childhood years Although older chil dren with this syndrome appear to tolerate large oral loads of calcium (6 11 13) the data presented here show their inability to dispose efficiently of an intravenous load

What are the possible explanations for the aberrant behavior of the subjects with the in fantile hypercalcemic syndrome? Under nor mal circumstances the relief of induced hyper calcemia of this duration probably involves several mechanisms (*a*) increased *excretion* into the gut or urine (*b*) rapid uptake by bone and (*c*) a reduction in bone resorption rate Appropriate changes in any of these mecha nisms could bring about a slowing of the rate of fall of serum calcium

The data of Table 1 show that *urine* excre tion accounts for only a minor portion of the total response and there is no difference be tween the "hypercalcemic" and control subjects

Another possibility is that the skeleton of the hypercalcemic subjects is more dense and therefore accepts calcium less readily from extracellular fluid It is known that skeletal density was increased in two of our patients in infancy but roentgenograms of the skull vertebrae and long bones did not appear abnormally dense in any of the patients at the time the calcium infusion studies were done Furthermore the 2 patients with documented hypercalcemia early in life had been maintained on very low calcium diets (less than 200 mg daily) for several months during infancy without the occurrence of hypocal cemia and none of the subjects have shown fasting hypocalcemia which suggests that the rate of calcium mobilization from the skeleton is adequate

The most likely explanation is a failure of the bone resorption rate to decline appropri ately in response to the calcium infusion It is known that a rise in serum calcium is normally accompanied by a reduction in parathyroid ac tivity *and* a release of thyrocalcitonin from the thyroid and that *both* phenomena lead to a prompt decrease in bone resorption rate (10 15 18) If *either* of these hormone responses is defective the post infusion rate of fall in serum calcium should be diminished

This is probably the situation in athyreotic hypothyroidism for these patients should lack thyrocalcitonin (a hormone elaborated by the "C-cells of the thyroid which derive from the ultimobranchial body) and several authors (3 16 32) have offered this as an explanation for the slow post infusion fall in serum calcium which they observed in such patients Our re sults are comparable to theirs in this regard Further evidence for this hypothesis is the observation that thyroidectomized animals have a reduced tolerance to parathyroid extract administration (2) and to oral calcium loads even when thyroxine replacement is given (20 23 29) The very slow rate of post infusion fall in serum calcium seen in frank hypothyroidism (*Table 1 Fig 1*) could reflect the combined effect of lack of thyroxine and thyrocalcitonin on calcium kinetics a phenomenon suggested by the low rate of urinary calcium *excretion* It is of interest that there are several reports of hypercalcemia and osteosclerosis in human hypothyroidism (4 24 25 27 31)

Our patients with the hypercalcemic syndrome however had none of the clinical signs of hypothyroidism and the serum protein bound iodine and the bone age (as measured by wrist roentgenograms) were normal in all

It may be claimed that patients with the infantile hypercalcemic syndrome have a form of autonomous hyperparathyroidism, in which parathyroid hormone release is not inhibited by superimposed elevations in serum calcium Evidence against this possibility includes a report of normal parathyroid glands at autopsy (26) and the fairly prompt fall in serum calcium when cortisone is given to infants in the hypercalcemic phase of the syndrome (14 28) None of our patients had abnormalities of serum phosphorus or alkaline phosphatase or characteristic findings of hyperparathyroidism on skeletal roentgenograms

It is tempting to speculate as Mitchell (22) and Chang et al (9) have done, that these patients are lacking in thyrocalcitonin—either in its production by, or its release from the C - cells of the thyroid gland (12) As a consequence the normal inhibition of bone resorption which occurs with induced hypercalcemia is impaired, hence bone resorption continues unabated and serum calcium returns to normal much more slowly The lack of thyrocalcitonin could be congenital due to an absence or abnormality of the ultimobranchial cells of the thyroid, while the thyroxine producing portion remains intact

Munson & Gray (23) and Care (8) have recently re emphasized the role of thyrocalcitonin in guarding against perturbations in serum calcium concentration Extirpation of the ultimobranchial body in chicks results in a delay in return of serum calcium to normal following induction of hypercalcemia (7)

Reasons for normalization of serum calcium in untreated patients during later childhood must also remain conjectural A possible explanation is that the load of calcium delivered to the body fluids from the usual dietary sources is less in later childhood than in infancy the usual infant intake of vitamin D is much greater than that of the child on a per kilogram basis and infants are known to absorb 2 to 3 times more dietary calcium (as per cent of intake) than older children (17) Hence the body fluids of older children are subjected to smaller loads of calcium small enough to permit a normal serum calcium in the absence of certain homeostatic mechanisms so that the tendency to hypercalcemia can be elicited only by giving calcium intravenously

The age effect noted for the controls (Fig 1) deserves comment the older the child the slower the rate of fall in serum calcium This phenomenon is quite in keeping with the well known effect of age on the rate of fall of serum radiocalcium following injection of the isotope in experimental animals (30) and Alexander has reported that serum calcium returns to normal much more quickly in young animals after EDTA infusion (1)

SUMMARY

Intravenous calcium infusions (10 mg/kg in 1 hour) were given to older children with the severe form of the idiopathic hypercalcemic syndrome and to controls The average half time of the post infusion fall in the increment of serum calcium for the patients was 1 7 times that of the controls These children are unable to handle calcium loads in an efficient manner

We postulate that the prolonged half time values seen in the hypercalcemic subjects is due to a failure of the bone resorption rate to decline in response to the induced hypercalcemia The most likely explanation for this phenomenon is a deficiency of thyrocalcitonin A similar explanation has previously been offered to account for the prolonged half time values found by ourselves and others in patients with hypothyroidism

Of further interest is the finding that the half time values of the controls vary directly with age

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It may be claimed that patients with the infantile hypercalcemic syndrome have a form of autonomous hyperparathyroidism in which parathyroid hormone release is not inhibited by superimposed elevations in serum calcium Evidence against this possibility includes a report of normal parathyroid glands at autopsy (26) and the fairly prompt fall in serum calcium when cortisone is given to infants in the hypercalcemic phase of the syndrome (14, 28) None of our patients had abnormalities of serum phosphorus or alkaline phosphatase or characteristic findings of hyperparathyroidism on skeletal roentgenograms

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Reasons for normalization of serum calcium in untreated patients during later childhood must also remain conjectural A possible explanation is that the load of calcium delivered to the body fluids from the usual dietary sources is less in later childhood than in infancy the usual infant intake of vitamin D is much greater than that of the child on a per kilogram basis and infants are known to absorb 2 to 3 times more dietary calcium (as per cent of intake) than older children (17) Hence the body fluids of older children are subjected to smaller loads of calcium small enough to permit a normal serum calcium in the absence of certain homeostatic mechanisms so that the tendency to hypercalcemia can be elicited only by giving calcium intravenously

The age effect noted for the controls (Fig 1) deserves comment the older the child the slower the rate of fall in serum calcium This phenomenon is quite in keeping with the well known effect of age on the rate of fall of serum radiocalcium following injection of the isotope in experimental animals (30) and Alexander has reported that serum calcium returns to normal much more quickly in young animals after EDTA infusion (1)

SUMMARY

Intravenous calcium infusions (10 mg/kg in 1 hour) were given to older children with the severe form of the idiopathic hypercalcemic syndrome and to controls The average half time of the post infusion fall in the increment of serum calcium for the patients was 1 7 times that of the controls These children are unable to handle calcium loads in an efficient manner

We postulate that the prolonged half time values seen in the hypercalcemic subjects is due to a failure of the bone resorption rate to decline in response to the induced hypercalcemia The most likely explanation for this phenomenon is a deficiency of thyrocalcitonin A similar explanation has previously been offered to account for the prolonged half time values found by ourselves and others in patients with hypothyroidism

Of further interest is the finding that the half time values of the controls vary directly with age

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Key words Infantile hypercalcemia, calcium thyrocalcitonin vitamin D

Our patients with the hypercalcemic syndrome, however had none of the clinical signs of hypothyroidism and the serum protein bound iodine and the bone age (as measured by wrist roentgenograms) were normal in all

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CYCLIC FLUCTUATIONS IN PLATELET COUNT, MEGAKARYOCYTE MATURATION AND THROMBOPOIETIN ACTIVITY IN CYANOTIC CONGENITAL HEART DISEASE

B GOLDSCHMIDT and RENÉE FONO

From the Second Department of Paediatrics Semmelweis University Medical School Budapest Hungary

In children with cyanotic congenital heart disease (CHD) thrombocytopenia is frequently observed (5 6 13) The cause may be decreased production or increased loss of platelets The latter concept is supported by the fact that the life of these platelets is shorter than normal (9) Data is lacking on the production of thrombocytes in this group of diseases Previously we had the opportunity to observe that in the blood plasma of cyanotic patients with congenital heart disease and thrombocytopenia there are thrombocytosis stimulating factor(s) (thrombopoietin) (4)

The plasma factor stimulating thrombocytosis was first studied by Kelemen et al (7) Later it was found by Rák et al (14) that this protein like substance was localised in the beta globulin fraction of the plasma Its effect in animal experiments is well known given intravenously or intraperitoneally it increases the production of megakaryocytes and thrombocytes In the bone marrow there is an accumulation of megakaryoblasts and the number and size of the megakaryocytes are increased Maximal thrombopoiesis may be observed in the peripheral blood on the fifth day after giving active plasma (1, 8 11)

Concerning the spontaneous regulation of platelet production there are no observations on either healthy or diseased humans During a longer period we studied continuously the trend of dynamic changes in the number of thrombocytes the thrombopoietic activity of the plasma and the megakaryocyte system in patients with C H D

MATERIALS AND METHODS

Twelve children with CHD were studied (Table 1) There were 10 cases of tetralogy of Fallot and 2 cases of transposition of the great vessels Haematocrit values ranged between 52 and 86 per cent the arterial oxygen saturation ranged from 40 to 92 per cent The polyglobuly did not change significantly during the period of observation The observations were continued for 10 weeks

Blood samples were taken in the morning hours from antecubital veins of patients using untreated needles and plastic syringes 10 ml blood was added to 500 I U heparin The platelet poor plasma (PPP) was obtained by means of centrifugation of the blood for 20 min at 4 000 rpm at room temperature The plasma was separated immediately and transferred to siliconized glass tubes It was used either immediately or within a few days In the latter case we stored it at −20 C The plasma samples were not handled sterilely

The platelet count of the patient was determined from venous blood every second to fourth day according to the method of Fischer & Germer (2) with phase contrast microscope The platelet count was carried out in two Burker chambers by the blind method The average of values obtained was considered the true number of platelets

The thrombopoietin activity of the patient s plasma was determined in mice For each assay 5-6 white mice of both sexes used from the BALB/c strain which were 8 to 16 weeks old The animals received a standard diet of commercial pellets and water *ad libitum* After d termining the platelet count we in

Table 1 *Clinical and hematological details of patients*

Case no	Sex	Age (years)	Diagnosis	Hemoglobin (g/100 ml)	Hematocrit (%)	Aortic oxygen saturation (%)
1	M	5	Tr	17.2	65	71
2	F	4	T F	14.4	52	85
3	F	11	T F	19.4	73	82
4	F	7	T F	13.2	53	92
5	M	4	T F	16.2	62	70
6	M	4	T F	18.6	78	85
7	M	10	T F	24.8	73	52
8	M	7	T F	19.9	78	67
9	M	14	T F	21.1	74	67
10	M	10	Tr	22.0	86	40
11	F	4	T F	15.6	52	74
12	F	5	T F	17.3	56	71

T F = Tetralogy of Fallot
Tr = Transposition ventricular septal defect

jected the mice intraperitoneally with 0.2 to 0.3 ml of the test plasma. The platelet count was repeated 5–6 days after the injection. The mean percentage rise in platelet count was taken to indicate the thrombopoietic activity of the test plasma injected. The thrombopoietic activity was accepted as increased (positive reaction) only if the thrombocyte count—with not more than one exception—had increased by at least 30% compared with the initial value.

Megakaryocytes were studied with bone marrow smear obtained by sternal puncture after May-Grunwald-Giemsa staining (10). The giant cells were classified morphologically into three stages of maturation:

stage I megakaryoblasts and promegakaryocytes
stage II granular megakaryocytes with basophilic plasma not yet producing thrombocytes
stage III mature megakaryocytes with oxyphilic plasma and platelet formation

The megakaryogram was obtained by counting 50 or 100 giant cells. The intensity of thrombopoiesis in the bone marrow was expressed with the so called maturation index (M.I.)

$$\text{M.I.} = \frac{\text{stage I} + \text{stage II}}{\text{stage III}}$$

Under normal conditions the M.I. is below 1.0. If in the marrow the megakaryo/thrombocytopoiesis increases the number of the young precursors increases and the megakaryogram shows a deviation to the left: the M.I. rises above 1.0.

The statistical evaluation was made with the Student's t test. A p-value of less than 0.05 was considered significant.

RESULTS

With 4 cases (nos 2, 4 and 8) selected at random in Fig. 1 the trend of platelet count and thrombopoietic activity of the plasma in patients are presented. In the other cases similar observations were made.

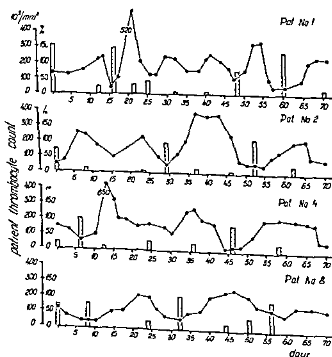


Fig. 1. Serial assays of platelet counts (●—●) and plasma thrombopoietin (▯) in patients with cyanotic congenital heart disease.

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left of the megakaryograms There was a significant positive correlation between the M I of the megakaryocyte system and the thrombopoietic activity ($p < 0.001$) (Table 4)

DISCUSSION

A number of human and animal experimental data support the observation that in several situations where the number of the platelets decreases below normal for one reason or an other there appears in the blood a protein like substance thrombopoietin Twenty four hours after the administration of human serum exhibiting thrombopoietic activity Krizsa et al (8) observed in the bone marrow and spleen of recipient mice the relative accumulation of the youngest megakaryocytes on the second and third day the absolute increase in the quantity of giant cells was observed After the third day the number of circulating platelets increases reaching the maximum of thrombocyte level on the fifth or sixth day The authors are of the opinion that the factor inducing thrombocytosis stimulates the differentiation of hemopoietic primordial cells in the direction of megakaryocytes

There are several conceptions on the feed back of the regulation of thrombopoiesis Following thrombocyte transfusions Odell et al (12) observed the depression of mega karyo/thrombocytopoiesis They presume that some specific inhibitory substance of circulating thrombocytes prevents the proliferation of megakaryocyte precursors After *in vitro* incubation of virulent platelets de Gabrielle & Penington (3) observed the disappearance of activity of the thrombopoietic plasma in thrombocytopenic donor animals Thus they are of the opinion that the thrombocytes themselves may adsorb the active substance and produce an apparently very simple mechanism of feed back

With examinations performed on patients with CHD we were able to observe the spontaneous dynamics in the regulation of the thrombocyte count Earlier it was demonstrated that some patients with CHD are thrombocytopenic (5 6 13) It was not known however that the number of platelets is changing cyclically or that normal and thrombocytopenic phases alternate with each other In the thrombocytopenic phase the plasma samples had a distinct thrombopoietic effect (4) Consequently the megakaryocyte precursors accumulate in the marrow and the megakaryopoiesis increases After a maturation period of few days the number of peripheral platelets rises and at the same time the thrombopoietic activity of the plasma disappears The megakaryocyte system shows again the normal usual qualitative rate

Gross et al (6) did not observe megakaryocyte deviation in cyanotic patients The evaluation of their study is difficult since they did not describe the method of evaluation of the giant cells

Kummer et al (9) demonstrated that in cyanotic patients the life of platelets is shorter than in normal individuals (3–4 days instead of 9–10 days) This would indicate that thrombocytopenia was a consequence of increased consumption of platelets Also the increase of the thrombopoietin activity and accumulation of the young or immature megakaryocytes in the marrow suggest a response to an increase either in peripheral destruction or platelet aggregation as a result of intravascular clotting (5)

Our findings indicate that in most patients with CHD the production of thrombocytes is normal though it may be increased in certain cases i e thrombocytopenia and is always regulated according to the requirements of the organism

SUMMARY

The authors observed that the thrombocyte count in children with CHD shows cyclic changes of 10 to 25 days In the state of thrombocytopenia the plasma exhibits a thrombopoietic effect and at the same time the megakaryocytic system in the bone marrow

Table 2 *Relationship of platelet count and thrombopoietin activity*

Platelet count (× 10³ per mm³)	No of determinations	Mean ( )	1 S D	p	Neg	Pos
< 120	32	+ 60	± 35	< 0 001	5	27
> 120	39	+ 22	± 29		31	8

Mean = Mean of the mean percentage rise in mouse platelet count
S D = Standard deviation

During the follow up the number of platelets changed periodically A single cycle lasted 10 to 25 days The extreme values were 20 000 and 500 000 thrombocytes/mm³ In general the platelet counts were below 300 000/mm³ The thrombopenic periods (< 120 000 platelets per mm³) lasted 3 to 15 days

On the first days of the thrombocytopenic state the plasma of all patients has a thrombopoietic effect (Table 2) The average increase in the platelet count of the mice was + 60 % (S D ± 35) In some cases we noted activity also with a normal number of platelets mainly in cases in which the arterial oxygen saturation was below 70 per cent After the platelet count became normal the plasma did not have important influence on the number of the platelets in the recipient mice Then the average increase in the platelet count of the animals was + 22 % (S D ± 29) The direction from the former values was statistically significant ($p < 0\ 001$)

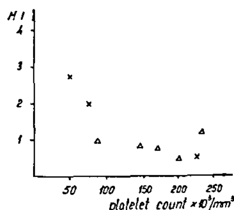


Fig 2 Number of circulating platelets and maturation index (M I) of the megakaryocyte system in bone marrow in patients with cyanotic congenital heart disease The thrombopoietin activity was positive (•) negative (Δ) and not examinated (×)

The deviation to the left of the megakaryogram in the bone marrow was observed during the thrombocytopenic phase of the cycle (Table 3) The megakaryoblasts and the promegakaryocytes showed a significant accumulation This is expressed by the M I which rose considerably above 1 0 With a normal number of platelets the M I is below 1 0 and the megakaryocytes and their precursors are no morphologic alterations There was a statistically significant difference between the two groups ($p < 0\ 001$) In Fig 2 the M I represents the number of the peripheral thrombocytes In the figure those cases are demonstrated where the thrombopoietic activity of the plasma could be astablished Except in one case the positive thrombopoietic activity went constantly parallel with the deviation to the

Table 3 *Relationship of platelet count of blood and the maturation index of the megakaryocyte system in marrow*

Platelet count (× 10³ per mm³)	No of determinations	Mean of M I	1 S D	p
< 120	6	1 9	± 0 68	< 0 001
> 120	8	1 0	± 0 84	

Mean of M I = Mean of the maturation index of patient s megakaryocyte system
S D = Standard deviation

Table 4 *Relationship of the maturation index of the megakaryocyte system in marrow and plasma thrombopoietin activity*

Maturation index	No of determinations	Mean ( )	1 S D	p
< 1 0	5	+ 21	± 13	< 0 001
> 1 0	6	+ 54	± 33	

Mean = Mean of the mean percentage rise in mouse platelet count
S D = Standard deviation

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INFANTS OF DIABETIC MOTHERS II

Acid–Base and Electrolyte Balance during the First 48 Hours after Birth

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In a previous paper the acid–base and electrolyte balance was studied in infants of diabetic mothers at caesarean section (16) It was found that these infants did not differ significantly in their acid–base and electrolyte balance from healthy infants *at birth*

After birth hypocalcaemia and hyperphosphataemia have been observed by several investigators (3 6 21) but on the other hand normal values of potassium sodium and chloride have been found (20) The aim of the present investigation was to study the changes in acid–base and electrolyte balance in infants of diabetic mothers *after birth* by taking blood samples at frequent intervals during the first 48 hours and to relate the results obtained to a group infants of healthy mothers

MATERIAL AND METHODS

Mothers

Eight diabetic mothers and their infants were compared with eight healthy mothers and their infants The diabetic mothers (DM) were 22 to 38 and the healthy mothers (HM) 18 to 40 years old All mothers were delivered by caesarean section the DM at 37 to 38 weeks and the HM 37 to 40 weeks of pregnancy Five mothers in each group were primiparous The indications for caesarean section in HM were cephalopelvic disproportion in 6 cases uterine inertia in 1 case and in 1 case the operation was performed because the mother was an elderly primigravida The DM were treated with insulin and had had diabetes for 7 to 18 years Before pregnancy 4 had retinopathy and 1 nephropathy during pregnancy 4 developed toxaemia and 1 hepatosis Throughout pregnancy the diabetes was well controlled within the limits for blood sugars accepted in modern treatment of diabetic pregnancy (11) in all cases except one who had marked hyperglucosaemia and glucosuria in early pregnancy The severity of diabetes in seven DM corresponded to cla s C and D in White s classification (18) and in one DM to class F All mothers received 5 5% glucose infusion during operation Before the study informed consent was obtained from both parents

Infants of diabetic mothers (IDM)

The eight infants had birth weights ranging from 1 950 to 3 930 g ($\bar{x}$ = 3 110 g) Two infants had birth weights below the 10th percentile and one above the 90th percentile for gestational age (4 10)

The condition at birth was satisfactory in all cases but during the first week of life the following symptoms were observed One infant developed mild respiratory distress another hyperbilirubinaemia, a third had a single low glucose value five infants were hyperexcitable and three had a weightloss exceeding 10 of birth weight

Infants of healthy mothers (IHM)

These infants had birth weights ranging from 2 450 to 4 170 g ($\bar{x}$ = 3 080 g) and within the normal range for gestational age A short episode of foetal bradycardi was registered in one infant whose condition at birth was normal Two other infants had initial Apgar scores of 7 but 5 minutes after birth the condition was satisfactory in all infants

The procedure immediately after birth was the same in both groups and the umbilical cord was clamped after cessation of arterial pulsations (late clamping) The postnatal care differed in the two groups the IDM were placed in incubators during the first days of life the IHM only during the first 2 hours The IDM obtained intravenous fluid therapy

shows a deviation to the left In the presence of a normal platelet count the thrombopoietic activity of the plasma disappears, the marrow megakaryocytes do not show morphologic alterations The intensity of platelet production changes according to the peripheral requirements

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INFANTS OF DIABETIC MOTHERS II

Acid–Base and Electrolyte Balance during the First 48 Hours after Birth

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In a previous paper the acid–base and electrolyte balance was studied in infants of diabetic mothers at caesarean section (16) It was found that these infants did not differ significantly in their acid–base and electrolyte balance from healthy infants *at birth*

After birth hypocalcaemia and hyperphosphataemia have been observed by several investigators (3 6 21) but on the other hand "normal values of potassium sodium and chloride have been found (20) The aim of the present investigation was to study the changes in acid–base and electrolyte balance in infants of diabetic mothers *after birth* by taking blood samples at frequent intervals during the first 48 hours and to relate the results obtained to a group infants of healthy mothers

MATERIAL AND METHODS

Mothers

Eight diabetic mothers and their infants were compared with eight healthy mothers and their infants The diabetic mothers (DM) were 22 to 38 and the healthy mothers (HM) 18 to 40 years old All mothers were delivered by caesarean section the DM at 37 to 38 weeks and the HM 37 to 40 weeks of pregnancy Five mothers in each group were primiparous The indications for caesarean section in HM were cephalopelvic disproportion in 6 cases uterine inertia in 1 case and in 1 case the operation was performed because the mother was an elderly primigravida The DM were treated with insulin and had had diabetes for 7 to 18 years Before pregnancy 4 had retinopathy and 1 nephropathy during pregnancy 4 developed toxaemia and 1 hepatosis. Throughout pregnancy the diabetes was well controlled within the limits for blood sugars accepted in modern treatment of diabetic pregnancy (11) in all cases except one who had marked hyperglucosaemia and glucosuria in early pregnancy The severity of diabetes in seven DM corresponded to class C and D in White s classification (18) and in one DM to class F All mothers received 5 5 % glucose infusion during operation Before the study informed consent was obtained from both parents.

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The eight infants had birth weights ranging from 1 950 to 3 930 g ($\bar{x}$ = 3 110 g) Two infants had birth weights below the 10th percentile and one above the 90th percentile for gestational age (4 10)

The condition at birth was satisfactory in all cases but during the first week of life the following symptoms were observed One infant developed mild respiratory distress another hyperbilirubinaemia, a third had a single low glucose value five infants were hyperexcitable and three had a weightloss exceeding 10% of birth weight

Infants of healthy mothers (IHM)

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The procedure immediately after birth was the same in both groups and the umbilical cord was clamped after cessation of arterial pulsations (late clamping) The postnatal care differed in the two groups the IDM were placed in incubators during the first days of life the IHM only during the first 2 hours The IDM obtained intravenous fluid therapy

Table 1 *Daily electrolyte intake (mEq/kg body weight)*

	Volume (ml/kg b w)	Potassium	Sodium	Calcium	Magnesium	Chloride	Phosphorus	Kcal
IDM								
First day								
glucose fructose i v	32 8	0 39	1 14	0 11	0 09	1 63	—	—
glucose orally	4 5	—	—	—	—	—	—	—
breastmilk orally	10 5	0 14	0 07	0 04	0 11	0 12	0 02	—
Total	47 8	0 53	1 21	0 15	0 20	1 75	0 02	19
Second day								
glucose fructose i v	21 3	0 54	1 11	0 16	0 12	1 88	—	—
glucose orally	—	—	—	—	—	—	—	—
breastmilk orally	33 5	0 47	0 23	0 13	0 37	0 40	0 07	—
Total	54 8	1 01	1 34	0 29	0 49	2 28	0 07	28
IHM								
First day								
glucose orally	3 2	—	—	—	—	—	—	—
Total	3 2	—	—	—	—	—	—	—
Second day								
breastmilk orally	19 4	0 28	0 14	0 08	0 22	0 24	0 04	—
Total	19 4	0 28	0 14	0 08	0 22	0 24	0 04	12

within the first hour with a solution containing 5% glucose 5 fructose and electrolytes and early feeding was started 6 hours after birth The IHM remained at the maternity ward and were treated according to general routine with feeding started about 18 hours aft r birth As they did not obtain immediate intra venous fluid therapy nor early feeding a discrepancy between the intake of calorie and electrolytes of the two groups occurred (Table 1)

Blood sampling and analytical methods

At delivery blood was taken by puncture of the umbilical artery In IDM an umbilical artery catheter was introduced and blood samples were drawn hourly during the first 6 hours and at 24 and 48 hours In IHM samples were drawn by femoral puncture at 1 2 4 6 24 and 48 hours The sampling procedure and analytical methods have all been described in detail earlier (15 16) except for a modified method for determination of lactate Within 1 minute of samplin 60 μl blood was transferred to 60 μl ice cold perchloric acid for lactic acid determination according to Jacob on (8) The analytical error was ±3 0

RESULTS

The mean values and standard deviations for the constituents determined were grouped according to the time of sampling The results are presented in Figs 1–6 The differences in mean values at different time periods were

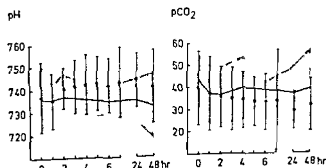


Fig 1 pH and Pco in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (In Figs 1–6 the mean values of IHM are connected with a line and the ±2 standard deviation limits represented by a shaded area)

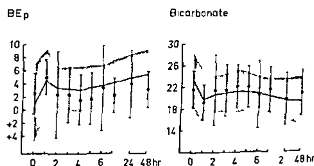


Fig 2 BE_p and bicarbonate in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (BE_p and bicarbonate in mEq/l)

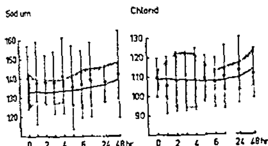


Fig 3 Sodium and chloride in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (Sodium and chloride in mEq/l)

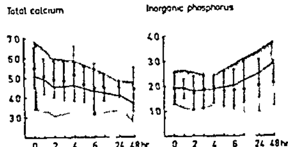


Fig 5 Total calcium and inorganic phosphorus in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (Total calcium in mEq l inorganic phosphorus in mM l)

tested for each constituents Probably significant differences were obtained for pH P_{CO} BE_p potassium total protein total calcium and inorganic phosphorus These differences and the non significant differences for these parameters are given in Table 2 Furthermore the significance of the slope of an estimated concentration line for everyone of the various components was calculated tested and presented in the text The significances are given as $*** = p < 0.001$ $** = 0.001 < p < 0.01$ $* = p < 0.05$

Acid–base balance

The pH was similar in both groups during the first hour after birth Later on the pH of IDM increased significantly ($F = 7.15^{**}$) to a level at the upper limits of IHM resulting in probably significant differences between the mean values at 4 6 and 24 hours The P_{CO} values were highest at birth and fell during the next hour In IHM the P_{CO} level remained fairly stable while it continued to fall significantly ($F = 21.04^{***}$) in IDM causing a probably significant difference between the groups at 24 hours of age The BE_p increased during the first hour and decreased the following hour in both groups In IHM the BE_p increased significantly thereupon ($F = 12.52^{***}$) while it remained fairly stable in IDM

Electrolytes glucose and lactate

At birth the *potassium* values were almost identical in both groups During the next 6 hours it tended to fall in IDM and to increase in IHM resulting in probably significant differences in mean values at 4 6 and 24 hours In IDM the plasma *sodium* values varied markedly and in IHM the sodium level in

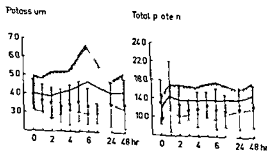


Fig 4 Potassium and total protein in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (Potassium and total protein in mEq/l)

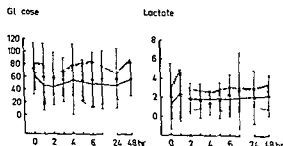


Fig 6 Glucose and lactate in arterial blood of IDM at various times after birth The mean values ±2 standard deviations are plotted against corresponding values of IHM (Glucose in mg 100 ml lactate in mEq/l)

Table 2 *The difference and their significance between the mean values of IDM and IHM at different sampling times*

Parameter	Sampling times hours after birth 0	1	2	4	6	24	48
pH	0 001 —	−0 005 —	0 029 —	0 064 *	0 071 *	0 093 *	0 085 —
P_{CO} mmHg	−4 1 —	0 1 —	−0 9 —	−5 3 —	−5 4 —	−7 9 *	−7 4 —
BE_p mEq/l	−1 6 —	−0 5 —	1 0 —	1 5 —	0 3 —	0 9 —	2 4 *
Potassium mEq/l	−0 06 —	−0 30 —	−0 36 —	−0 75 *	−1 34 *	−0 57 *	−0 67 —
Total protein mEq/l	1 2 —	−0 2 —	−1 0 —	−1 8 *	−2 4 —	−1 9 —	−2 3 —
Total calcium mEq/l	0 43 —	0 14 —	0 39 —	0 56 —	0 13 —	0 41 *	0 74 *
Inorganic phosphorus mM/l	−0 02 —	−0 02 —	−0 09 —	0 10 —	−0 17 —	−0 45 —	−0 80 *

The significances are given as *** = $p<0\ 001$ ** = $0\ 001<p<0\ 01$ * = $p<0\ 05$

creased slowly but significantly ($F=44\ 23$***) with increasing age The plasma *chloride* did not show any significant change or difference between the two groups The concentration of *bicarbonate* tended to decrease during the first hour after birth in both groups and to increase the next hour The concentration curve thereupon dropped in both IDM ($F=9\ 14$**) and IHM ($F=9\ 07$**) *Total calcium* decreased in IDM ($F=57\ 48$***) as well as in IHM ($F=71\ 90$***) during the observation period The mean concentration was somewhat lower in IHM with significant differences between the groups at 24 and 48 hours In IDM only 2 values out of 63 were below 4 0 mEq/l and no value was below 3 5 mEq/l In IHM no less than 14 out of the total 54 values were below 4 0 mEq/l and 7 out of these values were also below 3 5 mEq/l Five of the IDM were hyperexcitable and none of the IHM The level of ionized calcium calculated according to Zeissler (see Weisberg (17)) was almost identical in both groups After an initial decrease of 0 4 mEq/l the concentration remained stable at about 2 5 mEq/l during the first 48 hours of life The level of *total protein* increased promptly in both groups after birth After this initial adjustment the protein level remained fairly constant in IDM but increased in IHM ($F=35\ 60$***) The concentration of *inorganic phosphorus* was fairly unchanged during the first hours after birth but increased thereupon significantly in both IDM ($F=7\ 39$**) and IHM ($F=81\ 74$***)

The *glucose* level tended to decrease during the first hour after birth in both groups but it then remained fairly stable and at the same level during the next 2 days The *lactate* level rose during the first hour after birth whereupon it decreased next hour and stayed rather unchanged in IHM The lactate level showed no significant changes or differences in IDM

DISCUSSION

After birth and in the immediate postnatal period there are marked changes in the neonatal acid–base balance resulting in a combined respiratory and metabolic acidosis In newborns these changes usually disappear and the acid–base balance returns to normal at about 2 hours after birth (1 9) In the present study the variations in acid–base balance were almost identical during the first hour in both groups but later on the IHM had lower pH values In IDM the pH increased to a level at the upper limits of IHM and at the same time the P_{CO} decreased with increasing age This fall in P_{CO} was similar to that reported

by Prod hom et al (13) but occurred at a lower level The differences in acid–base balance between IDM and IHM may be caused by different care methods in the two groups The IDM were treated in incubators at neutral temperature zone with lower energy consumption intravenous fluid and early feeding while the IHM were kept in beds from the age of 2 hours onwards and almost without any caloric intake during the first day resulting in changes in the plasma residual anion fraction (Na + K + Ca + 2) – (Cl^- + HCO_3 + Prot) of the same nature as seen in starvation (14)

The levels of sodium and chloride were unchanged as found by Zetterstrom & Aberg (20) These authors reported the level of potassium to be within normal range but in the present study the potassium concentration of IDM tended to be lower than that of the IHM The major part of the potassium decrease was probably related to an increased glucose uptake occurring during the continuous infusion to these infants This assumed increase in glucose uptake could result in deposition of glycogen and potassium due to an increased insulin activity (5) Such mechanism has been proposed by Widdowsson (19) who also calculated that in rats fed on large amounts of sugar approximately 0 25 mEq potassium was deposited with each gram glycogen

The level of total calcium decreased gradually in both groups There was no difference in the decreasing values during the first 6 hours but thereupon the level continued to fall in the IHM while the concentration in IDM was unchanged which is not in agreement with other investigators In 1–4 days old IDM Zetterstrom & Arnhold (21) observed low average values of total calcium and found many infants to be hyperexcitable This symptom occurred in five IDM of the present study but could not be related either to the concentration of total calcium or calculated ionized calcium Furthermore several of the IHM had markedly low levels of total calcium but were not hyperexcitable

Concomittantly with the fall in total calcium the concentration of inorganic phosphorus increased in both groups In IHM this increase was higher and probably related to the lower caloric intake as "starvation may cause tissue destruction with release of phosphorus into the plasma (21)

It is well known that after birth the level of glucose in IDM declines rapidly to values below those observed in "normals and that the rate of this initial fall in glucose concentration is directly correlated to the maternal glucose concentration at delivery (2) In the present study no hypoglycaemia was recorded in the IDM except for a single glucose value below 20 mg/100 ml and the initial fall in glucose was similar in both IDM and IHM By maintaining acceptable glucose levels in DM throughout pregnancy the IDM were not exposed to a continuous maternal hyperglycaemia Furthermore the immediate glucose infusion therapy and the early feeding were factors thought to be related to the absence of hypoglycaemia in IDM

The temporary increase of lactate after birth in IHM was similar to that observed during the first minutes after birth in babies delivered by the vaginal route (12) The postnatal rise was probably not due to neonatal hypoxia but associated with increased respiratory work and crying

Early feeding of IDM with small volumes of glucose solutions beginning in the first hour after birth has been proposed by Gleiss (7) The managements of the IDM of the present study with intravenous fluid therapy within the first hour after birth and early feeding given 5 hours later were assumed to be associated with the low degree of metabolic acidosis and the low incidence of hyperbilirubinaemia and hypoglycaemia

SUMMARY

The acid–base and electrolyte balance was studied in 8 infants of well controlled diabetic mothers (IDM) and 8 infants of healthy moth

ers (IHM) during the first 48 hours after caesarean section

Microlitre methods were used for determination of pH total CO_2 potassium sodium chloride, total calcium, total protein inorganic phosphorus glucose and lactate

The acid–base balance did not differ significantly in IDM or IHM, although there were some variations within the groups In IDM the slow fall in P_{CO} with age resulted in a high pH and in IHM a late increase in metabolic acidosis was related to a low caloric intake

The concentrations of sodium and chloride were similar in both IDM and IHM but a slow increase of sodium occurred in IHM Plasma potassium tended to fall in IDM and to increase in IHM An initial concentration peak was seen for total protein in both groups and for lactate in IHM The concentration of total calcium decreased gradually and significantly during the first 2 days after birth and concomitantly the concentration of inorganic phosphorus increased significantly in both IDM and IHM

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PERSISTENT HYPERPHENYLALANINEMIA

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Blood phenylalanine mass screening of new borns has revealed variants of hyperphenyl alaninemia without or with only occasional phenylketonuria i e phenylketones in urine on a normal diet (4 5, 9 22) Having excluded hyperphenylalaninemia associated with both phenylalanine—transaminase and—hydroxyl ase deficiency and transient hyperphenyl alaninemia there remains a group of variants which Hsia (13) classifies as "persistent hyper phenylalaninemia This group constitutes be tween one third and one half of all instances of persistent elevations of phenylalanine in cluding classical phenylketonuria (13)

According to Berman et al (4) most patients with persistent hyperphenylalaninemia appear to develop normal intelligence without dietary treatment Thus recognition and delineation of this condition from phenylketonuria is im portant

The present investigation proposes that a 24-hours phenylalanine loading test is helpful in distinguishing infants with persistent hyper phenylalaninemia from those with phenyl ketonuria

MATERIAL

The data are obtained from 15 phenylketonuric chil dren 10 parents of these children 10 normal individu als and 3 children which we think should be classified as "persistent hyperphenylalaninemia" since they have hyperphenylalaninemia no phenylketonuria and after loading with phenylalanine no rise in serum tyrosine The diagnosis of phenylketonuria was confirmed by phenylalanine loading i e serum phenylalanine > 15 mg/100 ml (0 91 mmol/l) 48 hours after a loading test dose of 0 1 g L phenylalanine per kg of body weight

Case reports of the three children with persistent hyperphenylalaninemia are given below

This study was supported by grants from the P Carl Petersens Fund and the Research Committee of the Danish Mental Retardation Service (project no 93)

By phenylalanine tolerance we understand the daily intake of phenylalanine compatible with fasting serum phenylalanine values in the range of 5–10 mg/100 ml

CASE REPORTS

Case S M

Girl born April 1968 The family history did not show phenylketonuria or mental retardation Delivery normal birth weight 2 660 g the neonatal course normal She was fed on ordinary cows milk formula On the seventh day of life the blood phenylalanine level was 6 mg/100 mg by Guthrie test subsequently 3–12 mg/100 ml during the following weeks (Fig 1 S M) There was no demonstrable urinary excretion of phenylpyruvic acid or o-hydroxyphenylacetic acid

At the age of 4 months a low protein diet was instituted and subsequenty her phenylalanine tolerance increased up to 100 mg/kg/day

From the middle of the second year of life she has been on an unrestricted diet containing 1 500 mg phenylalanine per day Nevertheless her serum phenyl alanine values remained at the same level (Fig 1 S M)

The child was developing normally her EEG was normal and by psychometric test her IQ was 108 (October 1970 age 2 /₂ years)

Case F B R

Boy dizygote twin born April 1967 No family history of phenylketonuria mental retardation or consanguinity Apart from a threatened premature delivery in the seventh month the pregnancy and delivery were normal Birth weight 3 250 g The neo natal course was normal His physical development went quite normal his EEG was normal but later

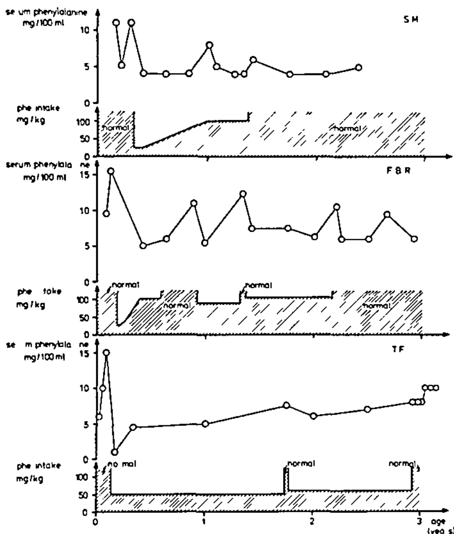


Fig 1 Serum phenylalanine concentration and phenylalanine intake in mg/kg/day of the 3 children with a high tolerance for phenylalanine but with absence of increase in serum tyrosine following phenylalanine loading Normal indicates unrestricted intake of phenylalanine

he proved to be slightly mentally retarded with IQ = 72 (October 1970 age 3 1/2 years) His twin sister has a normal phenylalanine loading test but is also mildly retarded with IQ = 77

The Guthrie test showed a blood phenylalanine level of 2 7 mg/100 ml in the first week of life gradually increasing to 16 mg/100 ml at the age of 6 weeks Serum tyrosine was normal The Phenistix test was negative Dietary treatment started at age 10 weeks with Lofenalac supplemented by vegetables and low phenylalanine bread from the fourth and eighth month of life respectively Throughout the first year of life his fasting values of serum phenylalanine were 5–12 mg/100 ml on a phenylalanine intake of 90 mg/kg/day

As the phenylalanine tolerance gradually increased to 100 mg/kg/day during the second year of life he was taken off the diet when 26 months old (Fig 1 F B R)

Case T F

Boy born June 1967 No family history of phenylketonuria mental retardation or consanguinity Pregnancy and delivery were normal Birth weight 3 500 g Neonatal course normal In the first week of life the Guthrie test revealed a blood phenylalanine of 3 2 mg/100 ml slowly increasing to 15 mg 100 ml at 4 weeks The Phenistix test was negative He was then placed on a low phenylalanine diet with Cymogran Vegetables and low phenylalanine bread was added from the 8th and 12th month of life respectively

Throughout the second year of life his phenylalanine tolerance increased to 60 mg/kg/day *After a* challenge with a normal diet for 3 days (approximately 1 500 mg phenylalanine per day) the fasting serum phenylalanine was still 7 mg/100 ml (Fig 1 T F) At nearly 3 years of age (November 1970) the low phenylalanine diet was abandoned and he was put on a normal diet with 2 000 mg phenylalanine per day His fasting serum phenylalanine values did not rise above 10 mg/100 ml (Fig 1 T F)

The physical development so far has been normal his EEG is normal and he is a well adjusted child of normal intelligence IQ = 112 (November 1970 age 3 /s years)

METHODS

Unless otherwise stated blood samples were withdrawn on fasting individuals in the morning (11)

L phenylalanine for loading was completely dissolved in 0 01 N HCl and the pure solution was given orally in a dose of 0 1 g per kg body weight Children below 3 years of age were tube fed The first meal was given 5 hours after the load test dose Blood samples were drawn at 1 2 3 4 and 24 hours after the loading In all children evidencing elevated serum phenylalanine samples were also taken at 2 3 4 5 7 14 21 and 28 *days* after loading The morning urine was collected before the load test and from then on the urine was collected separately for intervals of 0–6 hours and 6–24 hours after loading

Serum phenylalanine was determined by an adaptation of the fluorimetric method of McCaman & Robins (17) using 25 µl of serum The precision of the method estimated as $100/\bar{X}\sqrt{\Sigma(\bar{X}-X_i)}/N-1$ was 3 8 Serum tyrosine was determined by a microadaptation of the fluorimetric method of Udenfriend (24) using 150 µl of serum The precision of this method was 4 4% Either an Eppendorf photometer equipped with fluorimetric device for frontal fluorimetry or the Aminco SPF 125 spectrofluorimeter was used Thermostated cuvette holders were used with both instruments

Urinary *o m* and *p*-hydroxyphenylacetic acid was determined in 10 ml of lyophilized urine The residue was extracted by ethyl acetate after acidification and chromatographed in an isopropyl alcohol/aqueous ammonia/H O solvent as described by Armstrong et al (3) The absorbance after coupling with diazotized sulfanilic acid was measured at 432 nm with a Vitatron UFD 100 photometer equipped with a lin log converted UR 403 On the basis of the absorbance of aliquots containing 0 20–9 87 µmol/ml of *o*-hydroxyphenylacetic acid (FLUKA) the amount in µmol was calculated The precision of the method was 4 3% at 1 64 µmol/ml and 6 6% at 9 87 µmol/ml Creatinine was determined according to Popper s method (18) using the Boehringer modification Urinary phenylalanine and tyrosine was determined on a Technicon automatic amino acid analyzer employing 140 0 6 cm glass columns packed with the Technicon Type B (17 µ) resin A nine-chambered Autograd was used to supply the gradient elution buffer Sodium citrate buffers were prepared in the recommended manner The column was operated at 60 C

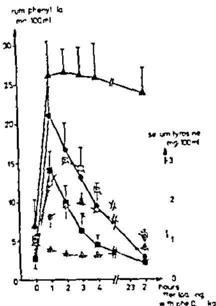


Fig 2 Serum phenylalanine and tyrosine concentrations within 24 hours after a L phenylalanine load of 0 1 g per kg body weight The points represent the mean with one standard deviation from studies of 15 children with P K U (▲) 10 parents of these children (●) and 10 normal individuals (■) Open signature and dotted lines indicate serum tyrosine concentrations

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L *phenylalanine loading*

When completely dissolved L phenylalanine was given orally to normal individuals or parents of phenylketonuric children the load of 0 1 g phenylalanine per kg of body weight was eliminated within 4 hours and associated with an increase in serum tyrosine reaching a maximum in normal individuals after 2 hours and in heterozygotes after 3 hours or more (Fig 2) In contrast phenylketonurics take about 14 days to eliminate this load Their serum tyrosine decreases slightly during the first 4 hours after the phenylalanine load (Fig 2)

The response of 3 children expected to have persistent hyperphenylalaninemia to the phenylalanine load appeared to be the same as in phenylketonurics i e no increase in serum tyrosine within 4 hours (Fig 3) Although this seems to indicate a lack of phenylalanine hydroxylation to tyrosine the serum phenylalanine concentrations returned to preloading levels within 24 hours (Fig 3)

Phenylalanine tolerance

As reported by Bickel (5) the daily phenylalanine tolerance per kg of body weight of children with phenylketonuria decreased within the first year of life The phenylalanine allowance of children with hyperphenylalanin

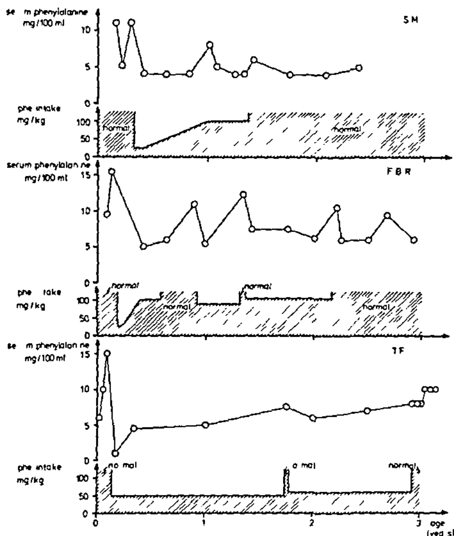


Fig 1 Serum phenylalanine concentration and phenylalanine intake in mg/kg/day of the 3 children with a high tolerance for phenylalanine but with absence of increase in serum tyrosine following phenylalanine loading Normal indicates unrestricted intake of phenylalanine

he proved to be slightly mentally retarded with IQ = 72 (October 1970 age 3 ¹/₂ years) His twin sister has a normal phenylalanine loading test but is also mildly retarded with IQ = 77

The Guthrie test showed a blood phenylalanine level of 2 7 mg/100 ml in the first week of life gradually increasing to 16 mg/100 ml at the age of 6 weeks Serum tyrosine was normal The Phenistix test was negative Dietary treatment started at age 10 weeks with Lofenalac supplemented by vegetables and low phenylalanine bread from the fourth and eighth month of life respectively Throughout the first year of life his fasting values of serum phenylalanine were 5–12 mg/100 ml on a phenylalanine intake of 90 mg/kg/day

As the phenylalanine tolerance gradually increased to 100 mg/kg/day during the second year of life he was taken off the diet when 26 months old (Fig 1 F B R)

Case T F

Boy born June 1967 No family history of phenylketonuria mental retardation or consanguinity Pregnancy and delivery were normal Birth weight 3 500 g Neonatal course normal In the first week of life the Guthrie test revealed a blood phenylalanine of 3 2 mg 100 ml slowly increasing to 15 mg 100 ml at 4 weeks The Phenistix test was negative He was then placed on a low phenylalanine diet with Cymogran Vegetables and low phenylalanine bread was added from the 8th and 12th month of life respectively

Throughout the second year of life his phenylalanine tolerance increased to 60 mg kg/day After a challenge with a normal diet for 3 days (approximately 1 500 mg phenylalanine per day) the fasting serum phenylalanine was still 7 mg/100 ml (Fig 1 T F) At nearly 3 years of age (November 1970) the low phenylalanine diet was abandoned and he was put on a normal diet with 2 000 mg phenylalanine per day His fasting serum phenylalanine values did not rise above 10 mg/100 ml (Fig 1 T F)

The physical development so far has been normal his EEG is normal and he is a well adjusted child of normal intelligence IQ = 112 (November 1970 age 3 ¹/₂ years)

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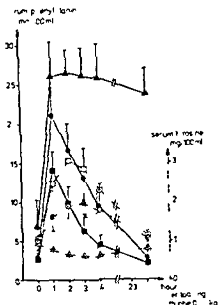


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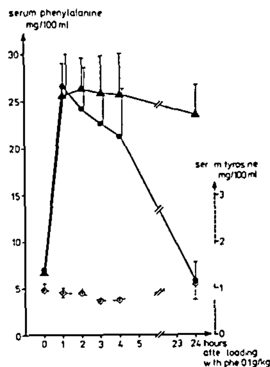


Fig 3 Serum phenylalanine (——) and tyrosine (- - -) (mean and one standard deviation) after L phenylalanine load (0 1 g per kg body weight) in 3 children (●) with persistent hyperphenylalaninemia and in 15 phenylketonuric children (▲) serum tyrosine concentrations of the children with persistent hyperphenylalaninemia

emia has to be restricted in order to keep fasting serum phenylalanine concentrations within 5–10 mg/100 ml (0 30–0 60 mmol/l), i e their tolerance is less than normal Phenylalanine tolerance corrected for age and body weight was correlated to the time necessary for eliminating a phenylalanine load (Fig 4) As will be seen, the 3 children eliminating the phenylalanine load within 24 hours (Fig 3) had a tolerance 5 to 8 times that of children with the prolonged course of elimination (5–20 days) which is characteristic of phenylketonuric children (Fig 4)

A retrospective study of these 3 children with high phenylalanine tolerance showed that unrestricted phenylalanine intake for periods of 2 months to 1 1/2 years did not elevate essentially the serum phenylalanine levels above values obtained during periods of a phenylalanine restricted diet, i e above 5–10 mg/100 ml (0 30–0 60 mmol/l) (Fig 1)

Excretion of o hydroxyphenylacetic acid phenylalanine and tyrosine

In the hyperphenylalaninemic children two possible mechanisms for phenylalanine elimina

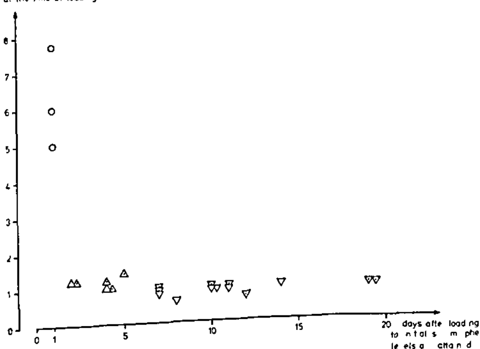


Fig 4 The relative phenylalanine tolerance in respect to age and body weight plotted against number of days required after a phenylalanine load to bring serum phenylalanine back to initial levels ○ indicates the 3 children with a high phenylalanine tolerance ▽ indicates 13 classical phenylketonuric children

tion other than *p*-hydroxylation to tyrosine, were explored namely *o* hydroxylation and excessive excretion of urinary phenylalanine. As shown in Tables 1 and 2 none of these mechanisms could be considered responsible. The excess excretion of o-hydroxyphenylacetic acid during the first 6 hours after phenylalanine load was no greater than in phenylketonuric children. The excess excretion of phenylalanine during the same period of time was similar to that of heterozygotes i.e. half the amount excreted by phenylketonurics though twice that of loaded normal individuals (Table 1 and 2).

Table 1 also includes the excess excretion of tyrosine during the first 6 hours after phenylalanine load. In the hyperphenylalanemic children tyrosine excretion during phenylalanine load is higher though not significantly than the slightly depressed excretion observed in the phenylketonurics (Table 1). The decrease in excretion of tyrosine during phenylalanine load in the phenylketonuric children correlates with the reduced serum tyrosine concentration during the load test (Fig. 2).

Serum tyrosine concentrations after overnight fasting

The observation indicating that children with persistent hyperphenylalaninemia may excrete more tyrosine during phenylalanine load than the phenylketonuric children suggests the possibility of a delayed phenylalanine hydroxylation to tyrosine in the former. In order to examine this possibility 97 consecutive values for serum tyrosine from 3 children with persistent hyperphenylalaninemia given an unrestricted diet were compared with 97 samples obtained from 10 children with phenylketonuria. The mean serum tyrosine concentration in the group of children with persistent hyperphenylalaninemia was 0.085 mmol/l (S.D. 0.016) whereas the mean tyrosine value in the group with phenylketonuria was 0.055 mmol/l (S.D. 0.014). This difference is significant at the 0.001 level. The dietary provision of tyrosine was comparable in the two groups. It should be mentioned however that the phenylalanine restricted diet (Albumaid®) provided the phenylketonuric children is enriched in tyrosine.

Table 1. *Excess excretion of urinary phenylalanine and tyrosine mean ($\bar{X}$) and standard deviation (S.D.) within the first 6 hours after phenylalanine loading test in normal individuals heterozygotes phenylketonuric children and children with persistent hyperphenylalaninemia*

		Phenylalanine (mmol/g creat.)		Tyrosine (mmol/g creat.)	
	n	$\bar{X}$	S.D.	$\bar{X}$	S.D.
Normals	10	0.173	0.065	0.085	0.045
Heterozygotes	9	0.435	0.244	0.091	0.061
Phenylketonurics	6	1.016	0.437	−0.105	0.110
Persistent hyperphenylalaninemia	3	0.560	0.170	0.013	0.027

Table 2. *Excess excretion of urinary o-hydroxyphenylacetic acid mean ($\bar{X}$) and standard deviation (S.D.) within the first 6 hours after phenylalanine loading test in normal individuals heterozygotes phenylketonuric children and children with persistent hyperphenylalaninemia*

		mmol/g creatinine	
	n	$\bar{X}$	S.D.
Normals	10	0.070	0.016
Heterozygotes	9	0.033	0.01[illegible]
Phenylketonurics	8	0.237	0.331
Persistent hyperphenylalaninemia	3	0.123	0.046

DISCUSSION

There is evidence for four alleles at the phenylalanine hydroxylase locus corresponding to 10 genotypes and hence 10 phenotypes (26). The several genetically determined modifications of the enzymes involved in the *p*-hydroxylation of phenylalanine to tyrosine may account for the different forms of hyperphenylalaninemia described (12). As pointed out by Hjalmarsson et al. (12) *enzyme kinetics and*

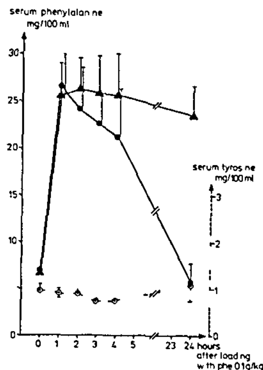


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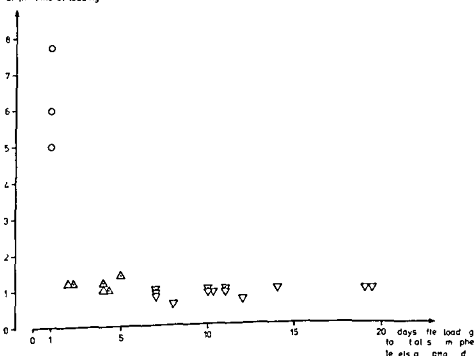


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y a route alternative to *p*-hydroxylation to yrosine e g *o*-hydroxylation to *o*-hydroxy henyl acetic acid 2) they may eliminate henylalanine by excessive urinary excretion r 3) they may be able to form small amounts f tyrosine which is immediately converted to lopamine noradrenalin and 3 5-diiodotyrosine on account of the great demand The present nvestigation seems to support the latter hypothesis This statement is based upon two findngs 1) the average serum tyrosine concentration in the fasting state was higher in the children with a high phenylalanine tolerance compared with phenylketonuric children receiving similar amounts of tyrosine in their diet (Albumaid[1]) 2) Children with persistent hyperphenylalaninemia showed a small increase in urinary excretion of tyrosine during phenylalanine load whereas the load depressed urinary tyrosine in the phenylketonuric children

The depressed excretion of tyrosine after a phenylalanine load in phenylketonuric children may simply reflect the concomitant decrease in blood tyrosine It is also possible however that a high concentration of phenylalanine in the blood contributes by interfering with amino acid transport across biological membranes (1 10 20) In that case the unaffected or slightly increasing tyrosine excretion in cases of persistent hyperphenylalaninemia may partly reflect the lower average level of blood phenylalanine during the first 6 hours after the test dose

The question whether children with persistent hyperphenylalaninemia possess some phenylalanine hydroxylase activity can only be definitely proved by estimating the hydroxylase activity in liver biopsy (15) Considering the risk involved in liver puncture in babies we have so far for diagnosis used the 24 hour phenylalanine tolerance test carried out at 3 months of age

Persistent hyperphenylalaninemia with negative or intermittently positive ferric chloride test may also reflect various degrees of partial phenylalanine hydroxylase deficiency In order to illustrate the heterogeneity Table 3 summarizes reported cases that have come to our knowledge of hyperphenylalaninemia with intermittent positive ketone test These variants are classified according to the course of serum tyrosine concentration after phenylalanine load Cases with an increase in serum tyrosine following loading are difficult to distinguish from the heterozygote state Cases without increase in serum tyrosine after loading with phenylalanine might be classified further according to the phenylalanine tolerance judged by the number of hours or days until the preloading serum phenylalanine level is regained after an oral load of completely dissolved L phenylalanine in an amount of 0 1 g per kg body weight

SUMMARY

The present investigation deals with extended phenylalanine loading studies on 15 phenylketonuric children 10 heterozygote carriers for that defect 10 normal individuals and three children with persistent hyperphenylalaninemia Those with persistent hyperphenylalaninemia had no increase in serum tyrosine following loading with phenylalanine suggesting lack of phenylalanine hydroxylation to tyrosine Nevertheless their serum phenylalanine concentrations reached preloading levels within 24 hours by a mechanism so far unexplained In contrast phenylketonurics take in average 14 days to eliminate this loading dose

Neither *o* hydroxylation nor excessive excretion of urinary phenylalanine could be considered responsible for the high rate of phenylalanine elimination in children with persistent hyperphenylalaninemia

It is proposed that a 24 hour phenylalanine loading test can be helpful in distinguishing infants with persistent hyperphenylalaninemia from those with phenylketonuria

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr Annalise Dupont and Dr Flemming Rosleff (The Pediatric

Table 3 *Reported cases of hyperphenylalaninemia arranged according to the phenylalanine loading te[st]*
PPA = Phenylpyruvic acid OHPAA = o-hydroxyphenylacetic acid

Ref list no	Patients n	Age when tested (years)	Initial se phenyl alanine (mg/100 ml)	Phenyl ketones in urine	Se tyrosine during load (mg/100 ml)	Phenyl alanine tolerance (mg/kg/day)	Clinical diagnosis	Latest IQ
(a) *With rise in serum tyrosine*								
14	1	4½	3–9	+PPA +OHPAA	0 5–1 55	?	Atypical PKU	64
21	1	33/52	12–50–40	+PPA +OHPAA	1 1–2 5	64–200	Temporary phenylketonuria	72
8	1	3	12 9	+PPA	2 5–5 5	30–100	Transient hyperphenylalaninemia	131
23	1	2/12	12–15	(+)PPA	0 67–0 91	?	Hyperphenylalaninemia PKU variant	83
16	6	7/12–4	20	−OHPAA	0 22–2 25	?	Atypical PKU	1 mildly retarded 5 norma[l]
(b) *Without rise in serum tyrosine*								
25	2	30	8 5	+PPA	unchanged	?	Phenylketonuria	102
		25	9 9	−PPA	unchanged	?	Occult PKU	83
2	1	13/12	15–18	—	unchanged	?	Atypical phenylketonuric hetero zygote	normal
19	1	10/12	12–20	+PPA	unchanged	25–50 unrestricted diet	Persistent hyperphenylalaninemia	normal
22	6	1/12–2	4–15	—	unchanged	unrestricted diet	Mild hyper phenylalaninemia	normal
27	9	3/52–6	6–20	—	unchanged or decreasing	45	Atypical PKU	7 normal 2 retarde[d]
12	1	33/360	10–16	—	decreasing	160–40	Mild PKU	?

genetic studies have to be made to characterize the different forms Until such studies have appeared the classification proposed by WHO (28) is recommended

1 classical PKU
2 classical PKU wih 2–3 times greater tolerance of phenylalanine than in the previous form,
3 transient hyperphenylalaninemia
4 persistent hyperphenylalaninemia

In the present investigation particular consideration has been taken to distinguish between classical PKU (group 1 above) and persistent hyperphenylalaninemia (group [4] above)

It is found that a 24 hour phenylalanin[e] loading test discriminates children having th[e] classical form of phenylketonuria from thos[e] with persistent hyperphenylalaninemia th[e] latter being able to eliminate the phenyl[-]alanine loading dose within 24 hours Th[is] finding is in agreement with a communicatio[n] by Blaskovics & Shaw (7)

Three alternative mechanisms may be re[-]sponsible for the high phenylalanine toleranc[e] of children with persistent hyperphenylalanin[-]emia 1) They may metabolize phenylalanin[e]

by a route alternative to *p*-hydroxylation to tyrosine e g *o*-hydroxylation to *o*-hydroxy phenyl acetic acid 2) they may eliminate phenylalanine by excessive urinary excretion or 3) they may be able to form small amounts of tyrosine which is immediately converted to dopamine noradrenalin and 3 5-diiodotyrosine on account of the great demand The present investigation seems to support the latter hypo thesis This statement is based upon two find ings 1) the average serum tyrosine concentra tion in the fasting state was higher in the children with a high phenylalanine tolerance compared with phenylketonuric children re ceiving similar amounts of tyrosine in their diet (Albumaid[R]) 2) Children with persistent hyperphenylalaninemia showed a small in crease in urinary excretion of tyrosine during phenylalanine load whereas the load depressed urinary tyrosine in the phenylketonuric chil dren

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GROWING PAINS

A Clinical Investigation of a School Population

JAKOB ØSTER and ARNE NIELSEN

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Only relatively few authors have expressed opinions and even fewer have undertaken investigations concerning this form of pain in the limbs which has been termed growing pains by both laymen and the medical profession from time immemorial (3) Only three works are available in the international literature which illustrate growing pains directly (2 5 7) This feature in association with the differential diagnostic significance of the condition and its presumed frequent incidence appears to justify a review of the relevant literature available (9) and an investigation of the incidence and clinical findings among school children The object of the present paper is to account for the latter

By means of ordinary clinical investigation including radiography and laboratory investigations it has not hitherto proved possible to reveal any recognizable etiology and in particular any evidence of rheumatic infection In order to establish relatively stable diagnostic directives we have established the following criteria from the international literature (1 8 9) and from personal experience

The symptoms consist of intermittent and frequently quite incapacitating pain localized deeply in the arms and/or legs in children and young people The pain is not articular Occasionally it is accompanied by sensations of restlessness but never by tenderness redness or local swelling Similarly pain in the legs is not provoked by walking and the gait is always normal The pain disappears in the morning

MATERIAL PROCEDURE AND OBJECT

During the school year 1968/69 one of the authors (JØ) questioned all of the children at the annual routine examinations of 2 178 school children in more or less the same way Do you have pain in your arms and legs— growing pains"?" When affirmative replies were obtained further questions were put concerning the localization, time and frequency of the pains and finally the degree of severity and their duration On the same occasion all of the children were questioned about abdominal pain and headache As part of the routine investigation, all illness and admissions to hospital during the preceding year were noted A routine clinical investigation was then undertaken Each child was weighed and measured and the ratio between weight and height was read from Døssings tables (4) although it was necessary to make estimations for some of the children whose heights exceeded those given by Døssing

The 2 178 children (1 062 boys and 1 116 girls) originated from rural and urban districts and from secondary general and academic curriculi and represented a cross-section of the population.

The ages of the children varied from 6 to 19 but the majority were in the age group 7–16 years

In order to illustrate the relationship between the problem of growth and growing pains, a control material of the same size as that with growing pains was formed by selecting as a control the next child for examination of the same age and sex but without growing pains

RESULTS

The total and percentage incidence of growing pains in both boys and girls are indicated

Table 1 *Absolute and percentage incidence of growing pains among 2 178 school children aged 6–19 years investigated in 1968/69*

Age (y)	Boys No	of		Girls No	of	
6	1	5	20 0	0	1	—
7	11	63	17 5	11	67	16 4
8	13	65	20 0	14	76	18 4
9	4	52	7 7	14	52	26 9
10	11	59	18 7	20	67	29 9
11	10	73	13 7	25	70	35 7
12	11	68	16 2	13	55	23 2
13	19	101	18 8	19	103	18 5
14	26	211	12 3	21	200	10 5
15	20	214	9 7	42	232	18 1
16	6	126	4 8	21	167	12 6
17	1	21	4 8	4	26	15 4
18	0	3	—	—	0	—
19	0	1	—	—	0	—
Total	133	1 062	12 5	204	1 116	18 4

Table 2 *The percentage incidence of growing pains abdominal pain and headache in 1062 boys and 1116 girls aged 6–19 years investigated in 1968/69*

Nature of pain	Boys	Girls	Total
Growing pains	12 5	18 4	15 5
Abdominal pain	9 6	14 8	1?3
Headache	18 3	22 8	20 6

in Table 1 The percentage incidence is shown graphically in Fig 1 In boys the incidence appears to be quite constant from the commencement of school age until the age of 13 years while in girls a maximum was found about the age of 11 years and thereafter a decrease but, nevertheless the incidence in girls at the age of 17 years was still higher (16%) than in boys (4%) The total incidence among all boys aged 6–19 years was 12 5% and among girls aged 6–17 years 18 4%

The percentage incidence of two other types of pain viz recurrent abdominal pain and headache appears from Table 2 It is apparent that the incidence of all three types of pain is greatest in girls and further that headache is the most frequent of these complaints This is followed by growing pains and finally recurrent psychogenic abdominal pain

The frequency with which growing pains occur in the individual child varied but in the majority 283 children, the pain occurred only occasionally and only 34 children were frequently inconvenienced by pain

The intensity of the pain was as a rule not particularly pronounced but 64 i e 22 3% of the 287 children who could express a definite opinion stated that the pain was severe Eight boys and 11 girls complained of frequent and severe pain and, in 20 out of 43 girls with severe pain this occurred in the evenings

A great proportion of the children (83) and this was identical for the two sexes stated that the pain occurred late in the afternoons and/or in the evenings In three boys and two girls the pain occurred at night and in all five of these cases severe pain was involved Otherwise pain occurred at varying times in 81 children i e a large group The majority of the children 126 out of the 337, were unable to give definite information about the time of occurrence of the pain

The localization of the pain was frequently stated to be in the musculature or at any rate in the deep structures of the thighs (152) behind the knees (73) calves (51) and upper arms

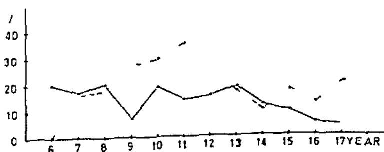


Fig 1 The percentage incidence of growing pains in 1 062 boys — and 1 116 girls -- aged 6–19 years from the school session 1968/69

(45) In addition five children complained of pain in the groins and four boys had back ache The pain was thus predominantly localized to the legs as a total of 292 out of 337 statements quoted these

None of the children who were included in the material suffered from organic disease of the muscles bones or joints as such conditions were excluded in advance according to the definition Only a very limited number of organic conditions were concerned

As mentioned previously the children with growing pains were also questioned about abdominal pain and headache and the results appear in Table 3 A total of 94 children or 28% suffered simultaneously from growing pains and headache and 74 children or 22% from growing pains and abdominal pain which is a greater percentage incidence of headache and abdominal pain than was encountered in the entire material (cf Table 2)

Out of the 332 children with growing pains for whom information is available about headache and/or abdominal pain the complaint is monosymptomatic (*i e growing pains* only) in the majority of cases viz 60 8 and multisymptomatic (i e that growing pains were accompanied by headache and/or abdominal pain) in 39 2 of cases which is however a minority This is apparent from Table 4 It is also observed that particularly in boys growing pains may occur as the only complaint while the two groups in girls were practically equal in size

Table 3 *Incidence of abdominal pain (A) and headache (H) in the 133 boys and 204 girls with growing pains*

A	H	Boys	Girls	Total
–	–	93	109	207
–		14	24	38
–	–	7	29	36
–	+	18	38	56
No information		1	4	5
Total		133	204	337
Abdominal pain		21	53	74
Headache		32	62	94

Table 4 *Distribution of pain into monosymptomatic or multisymptomatic in 332 children with growing pains*

Nature of complaint	Boys		Girls		Total	
	No		No		No	
Monosymptomatic	93	70 5	109	54 5	202	60 8
Multisymptomatic	39	29 5	91	45 5	130	39 2
Total	132	100 0	200	100 0	332	100 0

As regards conditions of growth information concerning height weight and weight/height ratio is available for 132 boys in the age group 6–18 years and 199 girls aged 7–17 years

The mean values of height weight and weight/height ratio respectively are calculated for each one year age group In Fig 2 these means are plotted against age not only for children with growing pains but also for the control group each sex separately The curves for children with growing pains and for the control group are much alike when children of the same sex are considered This likeness was tested statistically by comparing the variances with an F test and the mean values with a Student *t* test each sex and age group separately Out of the 66 F values 3 were significant as regards the 5% level the expected number being 3 3 One was further significant as regards the 1% level where 0 66 was expected Out of the 66 *t* values only one was significant as regards the 5 level This appears to be rather slight but an analysis of the distribution of the *t* values showed good agreement with that expected

It must therefore be accepted that growth as characterized by the three criteria height weight and weight/height ratio did not have any influence on the incidence of growing pains

DISCUSSION

In concluding these studies the authors consider it obvious that a clinically well-defined symptom complex is concerned and that re-

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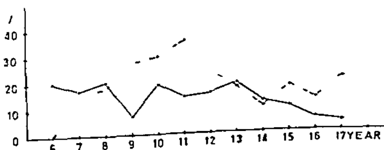


Fig 1 The percentage incidence of growing pains in 1 062 boys — and 1 116 girls – – aged 6–19 years from the school session 1968/69

frequent and severe growing pains In the present investigation this group comprized 64 or 22 3% which is scarcely 3% of all the children investigated i e a magnitude similar to that found by Naish & Apley

In the present investigation the pain was possibly of a milder character than found in previous works Hawksley (6) stated in 1939 that the pain of growing pains was more intense than in rheumatic infections and that approximately 45% of the children cried during the attacks In our investigation only a minority of children had severe and frequent attacks of pain and only very few stated that the pain was sufficient to make them cry

According to the definition employed here the pain is nonarticular Experience gained from the investigation indicates that articular pain is practically invariably of different significance than growing pains and further that growing pains are predominantly localized to the lower limbs These findings merely corroborate the findings of previous investigators (2 5 7 8) but may possibly be of value in illustrating the etiology and pathogenesis

Shapiro (8) was the first to emphasize that growing pains most frequently occur towards the close of the day or in the evening frequently after the child has fallen asleep Our own findings support this interpretation although numerous children mentioned varying times and strikingly many could only give uncertain information on this aspect

It is considered doubtful in advance that growth should cause pain In a material of 35 children aged 6–7 years with growing pains Brenning (2) could not demonstrate any differences in growth from a normal material The present investigation appears to exclude entirely the supposition that growth i e weight height or the weight/height ratio played any part in the etiology

It was not the object of the present study to investigate the etiology of the condition but it appears possible that growing pains just as psychogenic abdominal pain and "nervous headache indicate a special reaction form which occurs in a number of individuals and perhaps in certain families It may be emphasized that all three forms of pain cf Table 2 occur more frequently in girls than in boys and in addition cf Table 4 both headache and abdominal pain occur more frequently in children with growing pains than in the child population as a whole The problem of the relationships between these three forms of pain has been dealt with in more detail in another work (10)

SUMMARY

Growing pains are defined as non articular pain in the extremities without any demonstrable organic basis

In a representative school population consisting of 2 178 children aged 6–19 years growing pains occurred with an incidence of 12 5% among boys and 18 4% among girls Next to headache (20 6%) it is the most frequent pain in childhood (15 5%) the incidence of abdominal pain being only 12 3% Most often growing pains occurred only occasionally late in the afternoon or evening and were predominantly localized to the legs In most cases growing pains occurred without any other complaint

From this study it appears that growth i e height weight and weight/height ratio does not play any part in the etiology

Growing pains like recurrent abdominal pain and headache may be complaints which belong to a special emotional familial pattern

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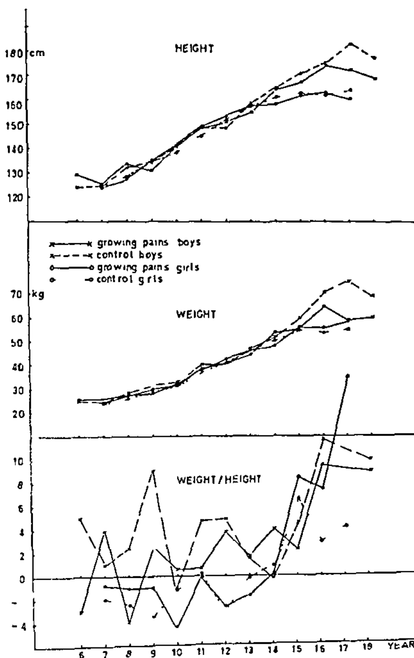


Fig 2 Height weight and weight/height ratio dependent on age in boys and girls with and without growing pains

search into the etiology and pathogenesis should be intensified Until these factors are elucidated it appears wise to retain the term growing pains but it should be borne in mind this is a diagnosis by exclusion which demands comprehensive differential diagnostic deliberations (8, 9)

After headache growing pains appear to be the most frequent form of pain in otherwise healthy children of school age and particularly in boys this appears to have a tendency to decline in incidence after the age of 13 years The work with which the present investigation best can be compared is that of Brenning (2) who found an incidence of 17% in children aged 6–11 years The total incidence of 15 5% in the present material is of a similar magnitude

The highest incidence was found by Hawksley (3) who found an incidence of 57 7% in a minority of his patient material but this apparently included all children who had experienced growing pains at one time or another Naish & Apley (7) found an incidence as low as 4 2% which is probably due to the fact that they only included children who had

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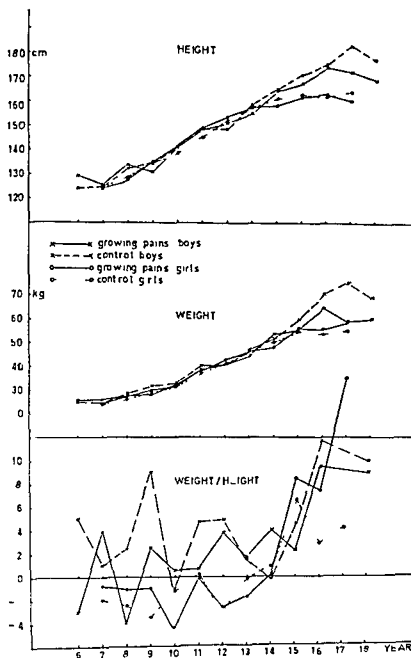


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HORMONAL TREATMENT OF UNDESCENDED TESTES AND IMMUNOGENIC PROPERTIES OF HCG

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Although human chorionic gonadotrophin (HCG) has been widely employed in pediatrics for the treatment of undescended testes its use is subject to criticism One of the possible disadvantages may be the hazard of inducing antibodies which could crossreact with endogenous pituitary gonadotrophins (8) and thus disturb the development of the testes

HCG is a potent antigen in rabbits or guinea pigs From the timing of HCG injec tions for the treatment of undescended testes an immunisation could easily be expected It is surprising that investigations on anti HCG antibodies in humans have not been reported all the more so since antibodies against In sulin HGH TSH Neurophysin Glucagon ACTH and even synthetic β^{1-4} corticotrophin (4) have been demonstrated in patients By the presence of such antibodies the biological ac tions of the respective hormones may be un affected blocked or prolonged In animals atrophy of the testes was observed after in duction of anti LH antibodies (6)

MATERIAL AND METHODS

For th present investigation we used a radioim munolo ical method A highly purified preparation of HCG (13 200 IE/mg) was used for iodonation (5) Patient s sera were incubated with the labelled hor mone for 24 to 48 hours Appropriate controls were set up with sera from untreated child en and with a rabbit anti HCG antibody respectively For separating antibody bound from free hormone we used the di oxan precipitation (9) 43 sera have been investigated from 39 children aged 3 to 13 years All patients had received the same preparation (Pregnyl Organon) at a dosage between 4 500 and 45 000 I U of HCG Blood samples were taken from a few days to several months after the last injection

RESULTS

As shown in Fig 1 no HCG antibodies could be detected in any of the 43 sera tested

DISCUSSION

The present negative result is surprising It cannot be explained by too small a number of investigations as the incidence of ACTH anti bodies is about 60% and HGH antibodies about 40 % of treated patients A methodo logical error could be excluded by an exten sive investigation of the technique used (7) A neutralisation of antibodies by compensa tory overproduction of endogenous gonado trophins should not completely mask any anti bodies in a radioimmunological study

It appears probable therefore that in spite of repeated administration of HCG anti bodies cannot be induced in humans

As a first explanation an immune tolerance against HCG could be discussed The fetus receives high doses of HCG at an early stage of gestation (1 2) before its immune competent system is developed The fetus continues to receive HCG during the entire pregnancy In all 53 newborns radioimmunoassay disclosed

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Key words Growing pains limb pains

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LIVER SCANNING AND LIVER FUNCTION IN CYSTIC FIBROSIS

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Paris France

Liver abnormalities have been frequently observed in cystic fibrosis (2 5 8 10 13 18 21 24) As described by Bodian on the basis of systematic histoanatomical examinations (2) the two most common lesions are (Fig 8) 1) Increase of the number of bile ductules and 2) focal biliary fibrosis Genuine multilobular cirrhosis (2 24) is much rarer being found in only 5 of the cases Hepatis lesions are almost always found at autopsy (2 25)

Results of hepatic function tests in 30 patients with cystic fibrosis were reported in a previous study (8) This study was done with isotopic exploration in order to specify the exact size of the liver its structure and its position in the abdomen (6 15 19 23 26) data concerning the hepatic blood flow and the reticulo endothelial system were also obtained (4 7 12 17 20 22)

MATERIAL AND METHODS

25 patients 7 males and 18 females aged 3 to 17 years all suffering from cystic fibrosis verified by means of sweat tests were examined by isotopic scanning (Table 1) Ten of these patients had hepatomegaly two of them patients 19 and 30 showed manifest signs of cirrhosis with portal hypertension hyperplenism and one of them had digestive haemorrhages Four patients died during the study and in three ca es histoanatomical examination was carried out

Investigation of oesophageal varices plasma protein studies and B S P clearances were made in addition to liver scans In 6 patients scanning was repeated and the find ngs were compared enabling us to follow the course of the disease

Isotopic technique

Colloidal gold (Au 198) was employed 16 times in 14 patients its half life is 2 7 days 2 μCi/kg body weight were injected Colloidal technetium sulfide (Tc 99m) was used 16 times in 12 patients The short half life of this isotope reduces radiation exposure to a minimum (17 19 23) Scan recording was performed 20 to 40 min after the injection by means of a scintillation cam ra (Pho-Gamma III Nuclear Chicago) The main advantage of a stationary detector is the short exploration time thus avoiding the difficulties of long immobilization required with the conventional method in dyspneic children The examination can also be carried out with the patient in a sitting position Three views were routinely studied 1) anterior view right and left hypochondrium 2) right lateral view 3) posterior view (4 15 23)

The costal margin the xiphoid process and the vertebral axis were chosen as anatomical landmarks Oscilloscope pictures were recorded on polaroid film and technical conditions standardized from one examination to the other A normal scan picture of the liver is characterized by a homogeneous distribution of the colloid in a liver of normal size of which the antero-inferior edge follows or protrudes slightly under the lower costal margin The isotopes used for this study in colloidal form have the property of being attached by the reticulo-endothelial Kupffer cells (15 22) these isotopes do not label the hepatocytes as seen with Rose B ngal labelled with radioactive iodine Under normal conditions only the hepatic reticulo-endotehial system is visualized due to the fact that the fixation is stable (11 15 17) There is no demonstrable dye uptake in the spleen or the vertebral bone marrow However Technetium sulfide particles are smaller in size and there can be an uptake of dye by the spleen (Fig 1) even in the absence of splenomegaly (7 12) Abnormal findings are characterized by hepatomegaly (Figs 2 3 and 6) uneven isotopic distribution with occasional filling defects (Fig. 4) and marked dye uptake by the reticulo-endothelial system of the spleen and bone marrow (Figs 5 and 7) (4 7 11 15 16 19 20 22 23 26)

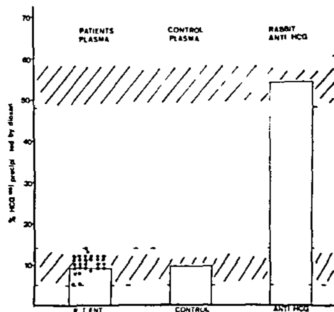


Fig 1 Lack of binding of HCG [125]J by plasma of HCG treated patients Binding of HCG [125]J by rabbit anti HCG (1/1000) ranging from 48–60 Non specific precipitation of HCG [125]J by control plasma ranging from 4–14% *Note* None of the individual values of plasmas from HCG treated patients (●) exceed the nonspecific precipitation

HCG which disappeared 3 to 4 days after birth (own unpublished observation) Furthermore there is evidence in man and in animals of an immune suppressive action of HCG itself (3 10)

A combined effect of immune tolerance and immune suppression could therefore explain the apparent lack of antigenicity of HCG in humans as shown by the present results Thus one of the possible arguments against a therapy with HCG is eliminated

SUMMARY

43 sera from 39 children treated with HCG for undescended testes have been investigated for HCG antibodies With a sensitive radio immunological method no antibodies could be detected in any of the patients Immune tolerance against HCG and/or the immune suppressive action of HCG are discussed as an explanation for the negative results

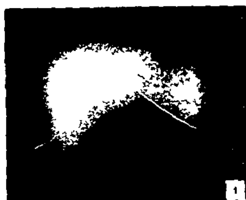
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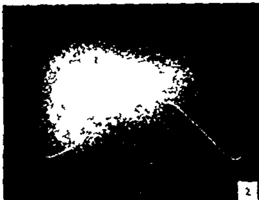
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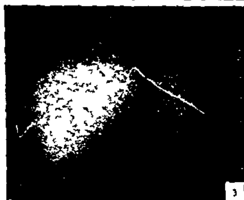
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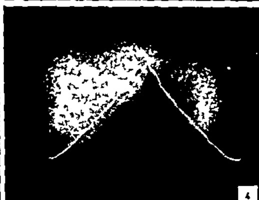
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Romergasse 8
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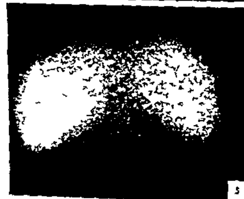
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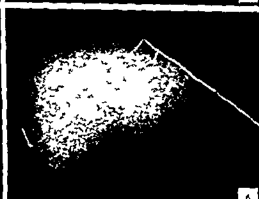

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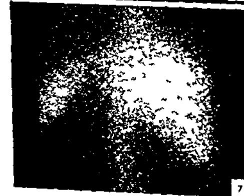

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Table 1 *Data on patients and comparison of results*

Case no	Colloid utilized	Sex	Age (y)	Pulm involvem	Clinical findings: Hepato megaly	Clinical findings: Spleno megaly	Proteins: Albumin (g/100 ml)	Proteins: Globulin (g/100 ml)	Proteins: Ratio A/G	BSP half life (min)	Oesophageal varices
Normal liver scan finding											
13	Tc 99m	M	6	2	–	–	4 16	1 2	1 3	4	–
16	Tc 99m	F	3	0	–	–	4	1 1	1 18	5	–
31	Au 198	M	5	2	–	–	4	1 3	1 2	5	–
Homogeneous hepatic hypertrophy											
1	Au 198 ( )	F	6	3	–	–	4 4	1 6	1 2	6 5	–
5	Tc 99m	F	9	1	–	–	4 9	1	1 8	4	–
7	Au 198	F	11	3	–	–	3 6	2	0 8	4	–
10	Au 198	F	9	1	+		4 9	1 2	1 6	6	Possible
12	Au 198	F	8	2	–	–	4	1 5	1 1	7	–
14	Tc 99m	F	4	1	–	–	3 6	1 3	0 98	3 5	–
23	Tc 99m	F	3	1	–	–	3 7	1 2	1 05	nal in 2 times	–
24	Au 198	F	6	2	–	–	4 1	1 6	1	4	–
25	Tc 99m	M	6	0	–	–	5 4	1	1 9	5	–
26	Au 198	F	11	2	–	–	4 4	1 4	1 32	7	–
27	Au 198	M	4	2	+	–	3 8	0 9	1 1	4	–
32	Au 198	F	13½	3	+	–	4	2 4	1	4	–
33	Tc 99m	F	5	2	+	–	4 3	1 3	1 36	5 5	–
Heterogeneous hepatic hypertrophy											
3	Au 198	F	6	2	–	–	3 6	1 2	1	4	–
4	Au 198	F	17	2	+	–	3 9	1 9	1	4	–
17	Au 198-Tc 99m	F	8	2		–	5	1 1	1 7	5	+
28	Au 198	F	11	3			3 6	1 5	0 9	3 5	–
Heterogeneous hypertrophy with spleen uptake											
18	Tc 99m	M	7	2	–	–	3 3	1 3	0 9	5 5	–
19	Au 198	F	4	0	+ (hypersplenism)	+	4 2	1 3	1 3	8	Possible
2	Tc 99m	F	10	3		±	3 1	1 1	1 6	5 3	–
Heterogeneous hypertrophy with spleen and bone marrow uptake											
2	Tc 99m	F	9	3			4 4	1 5	0 92	4 5	Possible
9	Tc 99m (+)	M	17	3	+	–	3 3	2 4	0 7	5 5	+
30	Tc 99m	M	7	1	+ (hypersplenism)	+	3 1	1 5	0 76	15	+ (bleeding)
32	Au 198 (+)	F	14	3			2 2	1 7	0 6	–	+

Grade of pulmonary involvement 0 minimal 1 – slight 2 moderate 3 – severe
Findings of patients 2 and 32 were recorded twice in this table

Fig 1 Normal liver scan with Technetium Tc 99m in a patient aged 3 years Uptake of the spleen without splenomegaly (patient 16)

Fig 2 Liver scan with Gold Au 198 homogeneous hypertrophy of the liver girl aged 6 years (patient 1)

Fig 3 Liver scan performed with Au 198 in a boy age 4 years homogeneous hypertrophy the liver is lowered by emphysema (patient 27)

Fig 4 Liver scan with Technetium Tc 99m in a girl age 11 heterogeneous hypertrophy Filling defect (patient 17)

Fig 5 Liver scan with Technetium Tc 99m on a boy age 8 years (cirrhosis of the liver) important spleen and bone marrow uptake and heterogeneity of the liver (patient 30)

Fig 6 Liver scan with Au 198 homogeneous hypertrophy of the liver in a girl aged 13 years with important pulmonary involvement and right cardiac hypertrophy (patient 32 anterior view)

Fig 7 Liver scan on the same patient (no 32) performed 10 days before death important hypertrophy heterogeneity spleen and bone marrow uptake (posterior view)

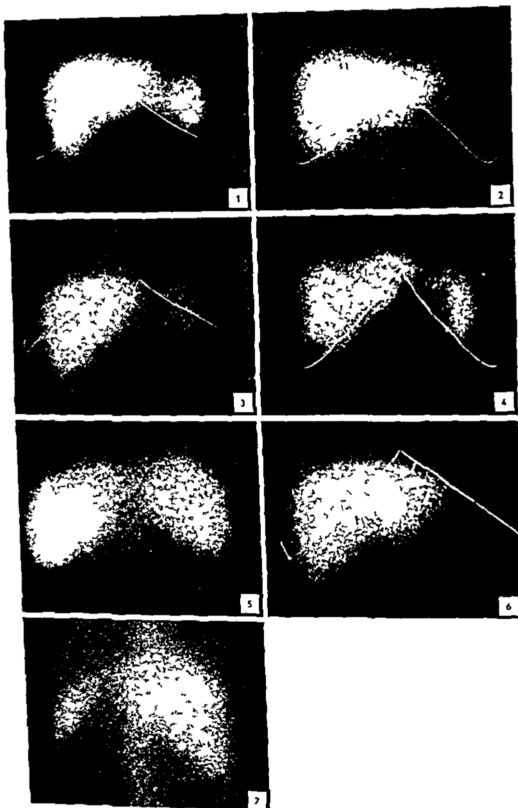

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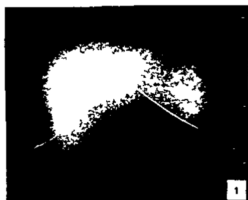
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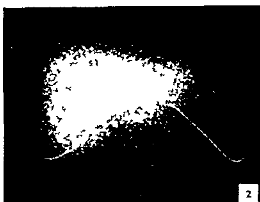
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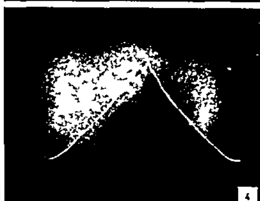
Fig 6 Liver scan with Au 198 homogeneous hypertrophy of the liver in a girl aged 13 years with important pulmonary involvement and right cardiac hypertrophy (patient 32 anterior view)

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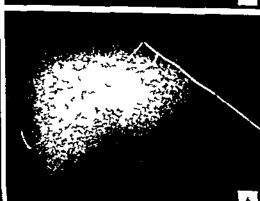

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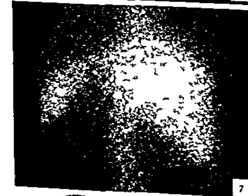

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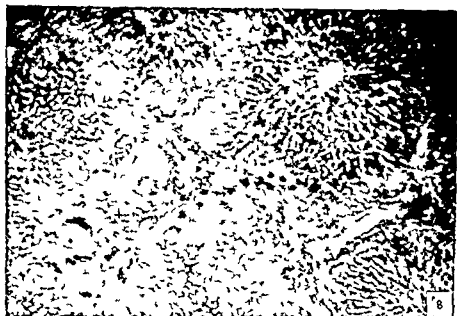
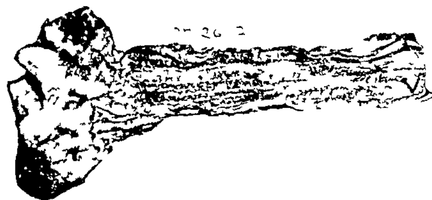


Fig 8 Section of the liver increase of bile ductules number and focal increase of their structures focal biliary fibrosis and cardiac liver (patient 32)

RESULTS

Only 3 of the 25 patients presented normal liver scans patients 13, 16 31 Abnormal aspects were found in the other 22 patients, hepatomegaly constituting the minimal criterion of abnormality Homogeneous distribution of dye was found in 13 patients Of these one had a liver enlargement due to cardiac decompensation (patient 32) and another (patient 1) died rapidly of acute pulmonary insufficiency Four children had a heterogeneous hypertrophy of the liver a 7-year old girl had a filling defect Repeated scans confirmed this defect as being comparable to those in Laennec s cirrhosis (4 9 16) Extrahepatic uptake was found in 6 patients in addition to hepatic hypertrophy and to heterogeneous distribution Uptake was purely splenic in 2 patients and splenic and vertebral in 4 patients In the latter group consisting of 1 patient with cirrhosis and hypersplenism and 3 patients with the most severe pulmonary symptoms, X ray revealed oesophageal varices Patient 2 had an improvement of her pulmonary status previously visible oesophageal varices disappeared improvement was also registered by liver scanning Two patients in this group died and histological examination revealed a biliary focal fibrosis as contrasted with normal hepatocytes (Fig 8) BSP clearances performed a few weeks before death had been normal



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Fig 9 Oesophageal varices on patient 32 a girl who died at 14 years of age Autopsy showed a cardiac liver and focal biliary fibrosis

DISCUSSION

Demonstration of hepatomegaly by isotope scanning in 22 of 25 patients with cystic fibrosis is not surprising Pathologists have already noted the usual hypertrophy of the liver along with its ptosis due to pulmonary emphysema and the resulting collapsed diaphragm

At the Paris Children s Hospital Watchi encountered hepatic hypertrophy at the autopsy of patients with no evidence of cirrhosis (25) Hepatic hypertrophy revealed by liver scanning is an in vivo demonstration of the frequent involvement of the liver in cystic fibrosis (2 5 8 13 18 21) It does not really disturb the liver function as shown by hepatic function tests A homogeneous distribution of the radiocolloid probably corresponds to the first stage described by pathologists i e the increased number of bile ductules (2 13 18) as seen in patient 1 Heterogeneous distribution is consistent with a more advanced stage of intrahepatic organic lesions such as focal biliary fibrosis (2) (Fig 8) The discontinuity seen in patient 17 is probably due to the confluence of several fibrous foci Two scintigrams performed with a 1 year interval showed no change in this zone Heterogeneous uptake also reflects local vascular changes due to fibrosis A decrease in hepatic blood flow can be aggravated by right ventricular insufficiency and thus brings about an increase of the splenic blood flow resulting in marked colloid uptake by the spleen (1 7) If the splenic reticulo endothelial cells are saturated dye uptake by the bone marrow reticulo-endothelial system may eventually be observed (1 26) Patients 9 and 32 died after prolonged pulmonary involvement They had splenic and bone marrow uptake and both had oesophageal varices (Fig 9) Autopsies showed moderate focal biliary cirrhosis without splenic lesions Scintigraphic indications along with oesophageal varices showed evidence of portal hypertension They nonetheless do not allow a discrimination between 1) cirrhotic hypertension due to portocaval shunts and 2) portal hypertension seen in cardiac liver and pulmonary insufficiency, due to pulmonary hypertension and portopulmonary anastomosis (1 3)

CONCLUSIONS

Morphologic studies of the liver by means of scintigraphy bring in vivo confirmation of the frequent involvement of the liver in cystic fibrosis This involvement can be of different types Comparison of radioisotope photoscanning of the liver clinical examination hepatic function tests and X ray examination of the oesophagus shows that scanning is a valuable method for exploring the liver thus permitting an accurate estimation of liver decompensation Scintigraphic examination is non traumatic It can be a useful adjunct in the supervision of the hepatic component and can contribute to the establishment of an accurate prognosis in patients with cystic fibrosis

SUMMARY

Examination of the liver in 25 patients with cystic fibrosis by means of isotope scanning standard hepatic function tests and X rays of the oesophagus showed only 3 patients to have a normal liver scintiscan 13 patients had evidence of hepatomegaly with homogeneous distribution of the isotope 4 patients had hepatomegaly and heterogeneous distribution and 6 patients had signs of extrahepatic uptake In this last group 2 patients had definite signs of cirrhosis of the liver and 4 had severe chronic pulmonary symptoms Two of these patients died and examination of their liver did not show cirrhosis but rather focal biliary fibrosis

Liver scanning on the whole confirmed data obtained through clinical biochemical and radiological examinations Scanning permits adequate visualization of the liver and the spleen and evaluation of the distribution of the isotope Scintigraphic examination is non traumatic It is a simple method for evaluating and following up the hepatic status of patients with cystic fibrosis and can be of great help in establishing their prognosis

ACKNOWLEDGEMENTS

The authors wi h to thank Dr Jacqu s Sauvegrain for his help in the radiologic part of this work and Professor Nezeloff Dr Iris Dr D may and Dr Jacqu line Gilly for pathological studies

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Key words Cystic fibrosis radio isotope scanning of the liver focal biliary fibrosis cardiac liver cirrhosis oesophageal varices

Acta Pædiat Scand 61 343–349 1972

ASPECTS OF PHARMACOLOGY OF GENTAMICIN IN NEWBORN INFANTS

JØRGEN BENT ANDERSEN NILS ROSDAHL and RENÉ VEJLSGAARD

From the Paediatric Department Rigshospitalet (Heads P Plum J C Melchior and B Friis Hansen) and the Institute of Medical Microbiology Department of Clinical Microbiology University of Copenhagen (Head M Volkert) Copenhagen Denmark

In infants the renal excretion of the aminoglycosides streptomycin and kanamycin is reduced being lower by two to four times in newborn and premature than in adults with normal renal function (1)

Gentamicin which chemically and biologically is closely related to the above mentioned antibiotics was introduced in 1963 (16) This antibiotic has been used in the field of paediatrics with rather favourable results although in small series (3 6 8 9 10) Gentamicin can cause ototoxic side effects (11) and it might be nephrotoxic in high concentrations although it has been claimed that this is not recorded (4) The available literature does not contain any reports on such side-effects in children during or after treatment with gentamicin ranging from 0 5 to 8 mg per kg per day

Like other aminoglycosides gentamicin is excreted by glomerular filtration (2) and the concentration of the antibiotic in urine of children is 80–100 times that of serum (12) However no more than 80 of the administered amount is recovered from the urine so a certain hepatic degradation might occur

The rate of glomerular filtration is lower in the newborn (15) which may suggest that the period of elimination is increased in these Approximately 30 % of the content of gentamicin in the blood in adults is bound to protein (2) so quantitative and qualitative changes in the serum proteins in newborn infants may change the degree of protein binding This might cause systematic errors in the results obtained by microbiological determinations of serum concentrations since pooled human serum from adults is employed as standard solvent

The object of this study has been to determine the half life of gentamicin in serum in newborn infants and to investigate whether long term treatment will result in accumulation of the drug

MATERIAL

The series comprised 25 newborn infants divided into two groups

Group 1 (Table 1) comprised 13 infants whose weight at birth ranged between 1 950 and 3 470 g their gestational age ranging from 34 to 42 weeks they received single doses of 1 or 2 mg of gentamicin by intramuscular injection The time of administration varied between the 1st and the 16th day after birth

Concentration of gentamicin in serum was determined in 5–6 samples of capillary blood drawn before and usually 1 2 4 6 and 12 hours after administration of gentamicin the half life was determined on the basis of the values obtained

In four of the children the total amount of protein and albumin in serum was determined immediately before the injection was applied

Group 2 (Table 3) comprised 10 newborn infants whose birthweight ranged from 900 to 3 700 g their gestational age ranging between 30 and 40 weeks These children received 2–5 mg of gentamicin per kg

ACKNOWLEDGEMENTS

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MATERIAL

The series comprised 25 newborn infants divided into two groups

Group 1 (Table 1) comprised 13 infants whose weight at birth ranged between 1 950 and 3 470 g their gestational age ranging from 34 to 42 weeks they received single doses of 1 or 2 mg of gentamicin by intramuscular injection The time of administration varied between the 1st and the 16th day after birth

Concentration of gentamicin in serum was determined in 5–6 samples of capillary blood drawn before and usually 1 2 4 6 and 12 hours after administration of gentamicin the half life was determined on the basis of the values obtained

In four of the children the total amount of *protein* and albumin in serum was determined immediately before the injection was applied

Group 2 (Table 3) comprised 10 newborn infants whose birthweight ranged from 900 to 3 700 g their gestational age ranging between 30 and 40 weeks These children received 2–5 mg of gentamicin per kg

Table 1 *Half life of gentamicin in serum in newborn infants after a single injection at different stages after birth in relation to weight age of gestation and serum protein concentrations*

Case no	Sex	Birth weight (g)	Gestation age (weeks)	Postnatal age for gentamicin administration (days)	Dose of gentamicin (mg/kg)	Half life of gentamicin in serum	Clinical diagnosis	Total serum protein (g/100 ml)	Serum albumin (g/100 ml)
1 (856/68)	M	1 950	40	1	1	6^h30^m	Gemellus low birth weight		
2 (82/69)	M	2 200	38	1	1	9^h30^m	Low birth weight		
3 (87/69)	F	2 350	40	1	1	5^h30^m	Low birth weight		
4 (823/68)	F	2 400	34	16	1	5^h	Low birth weight		
5 (356/70)	F	2 440	38	2	2	3^h15^m	Low birth weight dysmaturitas	5 3	3 00
6 (351/70)	F	2 450	38	2	2	7^h30^m	Low birth weight dysmaturitas	5 0	2 93
7 (792/68)	M	2 500	38	1	1	7^h	Low birth weight	5 3	
8 (358/70)	M	2 830	39	1	2	3^h50^m	Normal infant	6 0	3 82
9 (324/69)	M	2 990	39	1	2	8^h40^m	Morbus haemolyticus neonatorum		
10 (330/70)	F	3 080	38	3	2	3^h50^m	Morbus haemolyticus neonatorum	5 9	3 58
11 (860/68)	M	3 350	42	2	1	3^h30^m	Partus compl seq		
12 (747/68)	M	3 450	40	1	1	5^h50^m	Embryopathia diabetica	5 8	
13 (350/69)	M	3 470	38	6	2	6^h45^m	Morbus haemolyticus neonatorum		

Normal level of total serum protein 5–7 1 g/100 ml Normal level of serum albumin 2 5–5 g/100 ml

of body weight per day The intramuscular injections were applied twice a day i e at intervals of 12 hours

After commencement of therapy two samples of serum were obtained daily for 3 to 5 days One was taken prior to repeated injection of gentamicin and one was taken two hours after injection

As regards the first 8 children treatment was instituted during their first week of life The remaining two children whose weight at birth was 900 and 2 900 g respectively were treated when being 5 and 3 weeks old

During treatment the serum bilirubin level and the blood urea values were controlled daily the protein content in urine was determined by means of Albustix®

From 5 additional newborn infants not treated with antibiotics serum was obtained in order to compare pooled serum and infants serum (see methods)

METHODS

Blood sampling

Following local massage puncture was performed in the lateral edge of the heel by a sterile disposable lancet Excess rubbing of the heal after puncture was

avoided as far as possible The first drop of blood was wiped off upon which four capillary tubes were filled with blood (volume 70 μl each) and sealed with wax at one end Within one hour all samples were chilled to 4 C and taken to the laboratory in cooling containers (4 C)

Antibiotic

Gentamicin sulphate (586 μg base per 1 mg sulphate) Schering Corp

Medium

Bacto Antibiotic Medium 5 (Difco)

Test strain

A laboratory strain *Bacillus subtilis* in a spore suspension containing 10^{8}-10 spores per ml

Standards

Pooled human serum with the following concentrations 10 5 2 5 and 1 25 μg of gentamicin base per ml

A standard solution of gentamicin dissolved in the patients own serum was used for the examination of serum from patients nos 5 6 8 and 10 recorded in Table 1 The serum had been obtained before treatment was instituted

As regards the children nos 5 8 and 10 the routine laboratory method using pooled human serum as solvent for the preparation of standard solutions was employed to examine the standards prepared by the individual sera containing 1 and 10 μg gentamicin base per ml The same procedure was performed with sera from 5 additional infants (Table 2)

Technique

Petri dishes measuring 14 cm in diameter were filled with 50 ml of bacterium free medium after which they were left to solidify 15 ml of substrate containing 150 μl of the test bacteria suspension were poured over the base layer After drying at 37 C for 30–60 min filter paper discs (Schleicher & Schuell No 2668 diameter 6 mm) were placed on the surface Using sterile Carlsberg pipettes 20 μl of standards and samples were deposited on each disc Finally the plates were incubated at 37 C for 14–18 hours After incubation the plates showed confluent growth of the test bacteria except in the circular inhibition zones surrounding the discs the sizes of inhibition zones being conditional upon the gentamicin concentration in the samples and standards used

The zone diameter was read using a caliper or a projection apparatus which permitted a reading with an accuracy of 0 1 mm

On the basis of standard concentrations and corresponding zone diameters a curve was produced by plotting the zone diameter of standard solutions against log, of the concentrations The slightly curvilinear curve was drawn by means of a curve slide bar

Two plates were prepared for each determination of concentration each plate contained two discs with serum from one sample and thus quadruple determination of each sample was obtained

For a more detailed description of the method reference is made to V T Rosdahl et al (14) By calculation of the accuracy of the method these authors found a standard deviation of 0 15 within the range of measurement the scope of which ranges from the lowest standard concentrations to the highest concentration i e from 1 25 to 10 μg of gentamicin base per ml

Table 2 *Determination of pre fixed gentamicin concentrations in sera from children not treated with antibiotics*

	Pre fixed concentration of gentamicin in μg/ml serum		
Case no	1	5	10
5	1 4		9 5
8	1 2		10 4
10	1 7		16.5
I	1 2	5 6	11 5
II	1 1	6 5	10 5
III	0 7	6 0	12 0
IV	1 1	5 8	13 1
V	1 0	6 0	11.3

RESULTS

In Table 1 the individual half lives of gentamicin in serum are recorded If examinations were made within the first 24 hours of life of the patients concerned the average half life was found to be approximately 7 hours in the case of children whose weight at birth was below 2 500 g (nos 1 2 and 3) and being 6 hours in the case of children whose weight at birth had been above 2 500 g (nos 7 8 9 and 12) If the average is calculated for all children born immature the result is 6 hours and 15 min For those born at term and with birth weight equal or above 2 500 g the average is 5 hours and 40 min This difference however is not statistically significant and the deviation is rather large

In infants treated during their first week of life the initial dosage of 1 mg/kg body weight gave peak serum values between 1 9 and 3 2 μg/ml whereas dosage of 2 mg/kg resulted in peak serum levels from 4 0 to 7 6 μg/ml

Concentrations of total serum and serum

Table 1 *Half life of gentamicin in serum in newborn infants after a single injection at different stages after birth, in relation to weight, age of gestation, and serum protein concentrations*

Case no	Sex	Birth weight (g)	Gestation age (weeks)	Postnatal age for gentamicin administration (days)	Dose of gentamicin (mg/kg)	Half life of gentamicin in serum	Clinical diagnosis	Total serum protein (g/100 ml)	Serum albumin (g/100 ml)
1 (856/68)	M	1 950	40	1	1	6^h30^m	Gemellus low birth weight		
2 (82/69)	M	2 200	38	1	1	9^h30^m	Low birth weight		
3 (87/69)	F	2 350	40	1	1	5^h30^m	Low birth weight		
4 (823/68)	F	2 400	34	16	1	5^h	Low birth weight		
5 (356/70)	F	2 440	38	2	2	3^h15^m	Low birth weight dysmaturitas	5 3	3 00
6 (351/70)	F	2 450	38	2	2	7^h30^m	Low birth weight dysmaturitas	5 0	2 93
7 (792/68)	M	2 500	38	1	1	7^h	Low birth weight	5 3	
8 (358/70)	M	2 830	39	1	2	3^h50^m	Normal infant	6 0	3 82
9 (324/69)	M	2 990	39	1	2	8^h40^m	Morbus haemolyticus neonatorum		
10 (330/70)	F	3 080	38	3	2	3^h50^m	Morbus haemolyticus neonatorum	5 9	3 58
11 (860/68)	M	3 350	42	2	1	3^h30^m	Partus compl seq		
12 (747/68)	M	3 450	40	1	1	5^h50^m	Embryopathia diabetica	5 8	
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Normal level of total serum protein 5–7 1 g/100 ml Normal level of serum albumin 2 5–5 g/100 ml

of body weight per day The intramuscular injections were applied twice a day i e at intervals of 12 hours

After commencement of therapy two samples of serum were obtained daily for 3 to 5 days One was taken prior to repeated injection of gentamicin and one was taken two hours after injection

As regards the first 8 children treatment was instituted during their first week of life The remaining two children whose weight at birth was 900 and 2 900 g respectively were treated when being 5 and 3 weeks old

During treatment the serum bilirubin level and the blood urea values were controlled daily the protein content in urine was determined by means of Albustix®

From 5 additional newborn infants not treated with antibiotics serum was obtained in order to compare pooled serum and infants serum (see methods)

METHODS

Blood sampling

Following local massage puncture was performed in the lateral edge of the heel by a sterile disposable lancet Excess rubbing of the heal after puncture was

Pre-dose level of gentamicin (µg/ml)	Serum bilirubin (mg/100 ml)	Blood urea (mg/100 ml)	Clinical diagnosis
33	57	31	Low birth weight
?5	90	—	
?4	110	30	
0	105	14	
1	157	48	Low birth weight ABO incompatibility
14	173	—	
13	177	—	
1?	173	65	
1	171	40	Low birth weight hyperbilirubinaemia
16	155	28	
1?	139	—	
54	85	64	Diabetes matris hyperbilirubinaemia Pyuria
57	114	46	
—	et 191	—	
39	08	—	
25	et	16	
51		40	Cyanosis
29		—	
?4		?6	
0	170	0	Hyperbilirubinaemia
1	140	—	
1	—	31	
14	—	34	Cyanosis
14	2	—	
1	—	19	
49	5	44	Rhesus immunization
1	et 174	—	
1	et 118	55	
1		33	Low birth weight
1	×	—	
1		29	
1		—	
1		9	Rhesus immunization
0		—	
1		?0	

significant correlation between half lives and values of urea was recorded

In four patients (nos 5 6 8 and 10) prothrombin* and serum GO transaminases were all normal

It appears from Table 3 that pre-dose levels were initially high in patients in group 2 especially when the treatment had been instituted within the first postnatal days (nos 14 17 18 and 20) This tendency disappeared however within a few days and high pre-dose levels were not observed in the two children whose weight at birth had been 900 and 2 900 g respectively and who had been born during the 30th and 38th week of gestation in these cases treatment had been instituted during the 6th and 4th week of life respectively

The concentration of gentamicin two hours after repeated injection in one case (no 17) was 10 µg/ml but in none of the other determinations values above 7 7 µg/ml were found

Signs of reduced elimination were not observed in icteric newborn infants no matter

Table 3 *Pre dose of gentamicin during continuous treatment in relation to birth weight gestational age variation in dosage, and renal function*

Case no	Sex	Birth weight (g)	Gestational age (weeks)	Daily dosage of gentamicin (mg/kg)	Postnatal age for gentamicin administration (days)	Postnatal age for measurement (days)
14 (434/69)	F	950	31	2 0	1–5	2 3 4 5
15 (448/69)	F	2 350	34	3 4	4–8	5 6 7 8
16 (457/69)	M	2 400	35	4 2	5–7	6 7 8
17 (452/69)	F	3 200	37	5 0	1–7	2 3 4 5 6
18 (446/69)	M	3 200	40	3 8	2–5	3 4 5
19 (426/69)	M	3 250	40	3 8	4–8	6 7 8
20 (447/69)	M	3 510	40	4 0	1–5	2 3 4
21 (445/69)	M	3 700	40	3 2	4–8	5 6 7
22 (378/69)	M	900	30	3 3	34–40	35 36 37 38
23 (397/69)	M	2 900	38	2 7	18–29	20 21 22

e t = exchange transfusion — = no determination × = non icteric

albumin in patients nos 5, 6, 8 and 10 were normal and there was no correlation between the values and the half life

In the cases of children nos 5 8 and 10 where the children s own serum served for the preparation of standard gentamicin solutions the values recorded in Table 2 were found when determining these standards containing 1 and 10 μg per ml by the routine pooled human serum as solvent for standards Sera from 5 additional infants were investigated in the same way as the above mentioned using concentrations 10 5 and 1 μg gentamicin base per ml Generally the values were a little above the expected but almost all determinations were within the range of ± 2 times the standard deviation of the method except in the case of child no 10

In only one of the patients in Table 1 a slightly increased value of blood urea was recorded (no 6 43 g/100 ml) The other determinations were within normal ranges No

Pre-dose level of gentamicin (μg/ml)	Serum bilirubin (mg/100 ml)	Blood-urea (mg/100 ml)	Clinical diagnosis
3 3	5 7	31	Low birth weight
2 5	9 0	—	
.4	11 0	30	
0	10 5	14	
1	15 7	48	Low birth weight ABO incompatibility
1 4	17 3	—	
1 3	17 7	—	
1 ?	17 3	65	
1	17 1	40	Low birth weight hyperbilirubinaemia
1 6	15 5	28	
1 2	13 9	—	
5 4	8 5	64	Diabetes matris hyperbilirubinaemia Pyuria
5 7	11 4	46	
—	e t 19 1	—	
3 9	20 8	—	
? 5	e t	16	
5 1		40	Cyanosis
2 9		—	
2 4		26	
0	17 0	?0	Hyperbilirubinaemia
1	14 0	—	
1	—	31	
1 4	—	34	Cyanosis
1 4	2	—	
1	—	19	
4 9	25 2	44	Rhesus immunization
1	e t 17 4	—	
1	e t 11 8	55	
1		33	Low birth weight
1		—	
1		?9	
1		—	
1		9	Rhesus immunization
0		—	
1		0	

significant correlation between half lives and values of urea was recorded

In four patients (nos 5 6 8 and 10) prothrombin and serum GO transaminases were all normal

It appears from Table 3 that pre-dose levels were initially high in patients in group 2 especially when the treatment had been instituted within the first postnatal days (nos 14 17 18 and 20) This tendency disappeared however within a few days and high pre-dose levels were not observed in the two children whose weight at birth had been 900 and 2 900 g respectively and who had been born during the 30th and 38th week of gestation in these cases treatment had been instituted during the 6th and 4th week of life respectively

The concentration of gentamicin two hours after repeated injection in one case (no 17) was 10 μg/ml but in none of the other determinations values above 7 7 μg/ml were found

Signs of reduced elimination were not observed in icteric newborn infants no matter

whether the icterus was due to immunization (nos 15 and 21) or to a partially reduced liver function (simple hyperbilirubinaemia due to enzymatic immaturity) (nos 16, 17 and 19)

In two cases elevated blood urea and proteinuria were found during treatment Bacteriuria developed in patient no 15 a few days after treatment had been discontinued, but it subsided when treatment was resumed, parallel with a normalization of the renal function In patient no 17 treatment was initiated because of pyuria which subsided during treatment coincidentally with a normalization of the blood urea concentration

The blood urea levels were not influenced and proteinuria was not observed in any of the other infants

We did not observe any immediate side-effects after gentamicin treatment in our patients

DISCUSSION

In adults with normal renal function the half life of gentamicin is approximately 2 hours (13) Decreasing renal function in adults results in an increase in half life although no accurate correlation between dosage and serum creatinine has yet been established

In our investigations we found that the half-life in infants after the first 24 hours of life is increased by three times compared with that in adults The half life of gentamicin in low birth weight newborn was not increased statistically significant compared to that of normal birth weight infants Our findings are in accordance with previous investigations (3 10) According to McCracken & Jones (10) the differences between half lives in premature and full term infants are only evident for the first week of life and beyond this time the half lives were similar and approached that of adults

The serum protein concentrations are apparently not related to the length of the half life Neither jaundice seems to be of any importance A reduced renal function however diagnosed on the basis of an elevated blood urea level, seems to prolong the half life, in concordance with the fact that gentamicin primarily is excreted by glomerular filtration (2)

During the patients first week of life an increase in the pre dose level after commencement of treatment was observed However concentrations fell subsequent to treatment for a few days Whether this is due to age conditioned normalization of the kidney function or to adaption to gentamicin cannot be determined but in some of the children the urea concentration was decreasing which might support the former explanation The findings in patient no 22 might suggest that accumulation does not occur in children whose birth weight was very low if only their renal function is normal and provided they have attained the age of a few weeks

The post administration levels found in this series correspond favourably to the facts that gentamicin is distributed in the extracellular water (7) and that the extracellular space in infants is approximately 40% (5) In the treatment of premature as well as of fully developed infants after the first 24 hours of life administration of 2–4 mg/kg daily divided into two doses gave values two hours after injection within an acceptable therapeutic range although predose levels varied considerably (Table 3) Serum levels of gentamicin two hours after intramuscular injection might however not give sufficiently reliable estimates of the peak levels The vascularisation of the site of injection might vary considerably especially in these newborn infants Either numerous determinations of serum levels with narrow time intervals or establishment of the elimination gradient and subsequent extrapolation of this gradient to the time of injection might be indicative

The recommendations McCracken & Jones (10) that dosage schedules of 1 5 mg per kg per dose should be administrated twice a day to premature infants and 3 times a day to all other infants is supported by these investiga

tions However due to the fact that considerable variations in half life have been found determinations of gentamicin concentrations in serum are highly recommended in order to controlling dosages in such a way that toxic concentrations are avoided and therapeutic levels maintained

The employed micromethod of determination proved satisfactory in infants

The results obtained when comparing the activity of gentamicin dissolved in pooled human serum and in patient serum are not explained regarding child no 10 In the 7 other infants the differences is within the range of 2 times the standard deviation of the method The investigations of the serum from child no 10 could not be repeated as the child has been discharged from hospital The composition of serum protein could possibly be made responsible for the deviations found but until further evidence of individual differences in protein binding in infants serum is presented the discrepancy is considered to be due to technical errors

SUMMARY

A microbiological micromethod has been used for the determination of gentamicin concentrations in serum from 25 infants In 13 newborn infants whose birth weight ranged from 1 950 to 3 470 g the half life of gentamicin in serum varied from 3 h 30 min to 9 h 30 min A significant relation between half life and birth weight or gestation age was not demonstrable In 12 infants the rate of excretion was very low during the first few days of life judged by initial high pre-dose levels but after some days the excretion increased

Neonatal jaundice did not influence the excretion rate

Normally 2–4 mg gentamicin per kg per day divided in two or three doses could be given to newborn infants but determinations of gentamicin concentration in serum is the only criteria sufficiently strict to serve as basis for the determination of gentamicin dosage in newborn infants

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CASE REPORT

TRISOMY G/NORMAL MOSAICISM IN A MENTALLY RETARDED GIRL WITH SOME DYSPLASTIC FEATURES

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The effect of trisomy G/normal mosaicism is not quite clear Some mosaic individuals are similar to a mongoloid patient and others are quite normal, the latter has been the case with a few mothers of mongoloid children (5) In view of this variability we thought it of interest to report a case which differs from most of the earlier cases Our case seemed also to accord with the experiences of Kohn et al (3) that mental prognosis is not much better for mosaic dysplastic cases than for pure trisomy G mongoloids

CASE REPORT

S B 69 07 13 The patient a girl 20 months old is the only child of a 22 year old mother and a 22 year old father The parents were physically and mentally normal There was no consanguinity and no family history of congenital malformations The mother had had no miscarriages

Delivery was normal at term after a normal gestation Birth weight 2 940 g length 50 cm head circumference 33 cm The postnatal course was normal but the child showed some dysplastic features At five months of age she was admitted to the hospital because of a serious laryngotracheobronchitis She was then irritable but examination of CSF showed no cells and a total protein of 42 mg/100 ml There was no obvious mental retardation at that age Two months later she was observed to be deeply mentally retarded She had a muscle hypotonia Her legs were held maximally abducted She did not grasp could not sit without support and did not turn in bed Her mental development has since been very slow First when it was obvious that she was mentally retarded the chromosomal investigation was performed

Present condition

Physical examination showed a well nourished girl of normal stature (84 cm) and weight (11 9 kg) Cephalic index was 0 860 She could not sit up without support There was no sensory contact It was impossible to decide whether she could see but she seemed to hear a bell from 2 5 m distance She was apparently relaxed and showed no spontaneous activity The hair was somewhat curled her face broad eyes normal size and the palpebral fissures were not oblique (see Fig 1) Iris spots were not seen There was a midface retraction which is not usual in mongolism The ridge of her nose was flat The occiput was flat but there was no excess of neck skin The ears were small and dysplastic There was a joint hyperflexibility and the legs were held maximally abducted The teeth were small but otherwise normal

Radiological studies

X ray showed the heart and the chest to be normal as were the pelvic and hand skeleton Normal skeleton age Air encephalogram was normal

Laboratory studies

The galactose 1 phosphate uridyl transferase activity was normal 31 units per g Hb Electro-encephalogram at the age of 11 and 16 months showed episodic delta activity sometimes with spikes on the right side

Dermatoglyphic studies

According to Professor L S Penrose (personal communication) the dermatoglyphic pattern showed a strong suggestion of mongolism However the pattern was not grossly abnormal it could occur also with other conditions The chief points were that



Fig 1 En face photograph of the patient 16 months of age

ulnar loops occurred on all fingers except on one which had a radial loop (see Fig 2) (4) The patterns on the palm were quite normal The soles were lack in in pattern intensity as is common in most types of chromosomal aberration The rather small distal loops (less than 20 ridges) in the hallucal areas were consistent with a diagnosis of mongolism The dermato lyphics of the parents were not investigated

Cytogenetic studies

The chromosomes of the proposita were studied in blood on two occasions and in skin All the three in vestigations gave about the same result trisomy-G/

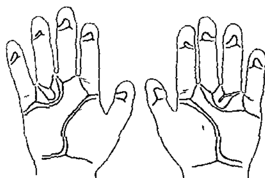


Fig 2 Dermatoglyphic patterns of right and left palms of the patient

Table I *Chromosome results on patient*

Tissue	Chromosome counts 46	47	Karyotype analysis
Blood I	47	3	All cells with 47 chromosomes had an extra G chromosome trisomy G
Blood II	30	5	
Skin	6?	10	

normal mosaicism with about 10 trisomy G cells (Table 1) There were no structural aberrations noticeable in the karyotype of the proposita Her parents had normal chromosomes no mosaicism In both 20 cells were analysed A further investigation was wanted but the parents refused to collaborate

DISCUSSION

Clinically this patient is not a mongoloid although she has some dysplastic features which are also met with in mongoloids small dysplastic ears and flat nasal bridge Other features are opposed to those usually found at mongolism Her features do not show the mongoloid smallness her hair is curled pelvic and hand skeletons are normal The dermatoglyphic pattern however is strongly suggestive of mongolism Penrose (4) has shown that dermatoglyphic features of mosaic individuals are often intermediate between those found in mongolism and in normals

Intermediate phenotypes are typical also for other chromosomal mosaicism cases—XO/XX mosaicism has a clinical picture ranging from that of a complete Turner s syndrome to that of a normal woman (1) XXY/XY mosaicism is not always connected with sterility but XXY karyotypes are (6) only C trisomy/normal mosaicism is compatible with postnatal life (7)

The reasonable explanation of this phenomenon is that the mixture of normal and abnormal cells gives a less marked abnormal development than in the purely trisomic or monosomic individuals

Kohn et al (3) and others have stated that the mental prognosis is not much better for mosaic dysplastic cases than for pure trisomy G mongoloids Johnson & Abelson (2) com

paring regular trisomy G translocation and mosaicism types of mongolism even found that those with mosaicism had a slightly lower intellectual ability Just as in the case of physical features, the mental development might be dependent on the proportion of trisomic cells present in the brain Therefore, different degrees of mental development would be expected from normal development to that found in mongoloids

The present patient demonstrates one source of error in this connection The slight dysplastic features not much resembling these found of mongolism did not indicate the need for a cytogenetic investigation until the mental retardation was noted The mental subnormality may be due to the chromosomal abnormality possibly aggravated by the serious infection at the age of 5 months or could even have some other explanation What decided the selection of this patient for cytogenetical investigation thus revealing her condition of mosaicism, was the *combination* of slight dysplastic features and obvious mental retardation This may be a common occurrence and the prospect of cytogenetic study of a child with mosaicism without mental retardation or only slight retardation is small At the other extreme are the normal individuals with trisomy G/normal mosaicism who are studied cytogenetically because of the birth of a mongoloid baby Mental development is not involved at this selection and the few parents with mosaicism that are found have also shown a reasonably normal development

If the above arguments are true there should also be found mosaic individuals with an intermediate degree of mental development That so few cases are on record may be simply because they are difficult to find

SUMMARY

A 20 month-old mentally retarded girl with trisomy G/normal mosaicism and slight dysplasia is described The mental prognosis for mosaic cases is discussed

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CASE REPORT

NEMALINE MYOPATHY

Report of Four[1] Cases and Review of the Literature

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Nemaline myopathy first described in 1963 by Shy et al (26) is characterized clinically by congenital nonprogressive or slowly progressive muscular weakness most prominent in the proximal muscle groups Histological preparations of a muscle biopsy reveal sub sarcolemmal aggregates of minute abnormal rod shaped or thread like structures Because of the thread like appearance of these struc tures under the light microscope the disease is called nemaline myopathy At the ultra structural level these rods seem to represent an accumulation of Z band material with axial and cross striae and in cross sections at high magnification they seem to have a crystal lattice structure Up to now at least 30 pa tients with this disorder have been published 19 of them females and only 5 patients with late onset (Table 1 and 2) Because there have been no previous reports of nemaline myo pathy in the Scandinavian countries we pre sent 4 cases diagnosed at the Children s Hos pital University of Helsinki in 1970

REPORT OF CASES

Case 1

J S born 28 12 63 is a 6 year old boy (Figs 1 2 3) He is the third of four children and there is no family history of muscle diseases The parents are not aware of any consanguinity Except for some bleeding gestation was uneventful and the mother did not notice any impairment in the movements of the foetus in utero Delivery was normal and the birth weight 4 100 g, Apgar score 6 at 1 minute and 7 at 15 minutes after birth The child was at first kept in an oxygen incubator because of drowsiness and it had difficulty in sucking Muscular weakness was observed from birth His physical development was slow he walked at 2 5 years and later needed splints because of foot drop His intelligence was normal He was followed up at a provincial central hospital No definite diagnosis was established but the disease seemed to be some nonprogressive myopathy He was also given speech therapy because of indistinct speech

The patient was admitted to the Children s Hospital University of Helsinki in March 1970 He was in a good general condition and his mental status was normal His height was in the 50th percentile growth curve but his weight remained between the 16th and 25th percentile curves His appearance was typically myopathic with slender build and accentuated lordosis and an elongated expressionless face and high palate The gait was waddling The speech was very indistinct and the voice high pitched The extremities were thin and atrophic with long phalanges The distal muscles of the extremities were weaker than the proximal ones The dorsal flexors of the feet were especially weak when he walked his feet dropped and turned to inversion He was not able to lift his head when lying on his back, because of the weak neck muscles In rising from the bed he first turned on his stomach then put his feet on the floor and lifted up his trunk by straightening his arms The deep tendon jerks were weak and symmetrical His condition was otherwise normal except for secondary findings due to poor muscle power Blood count, urinalysis skull X ray EEG and CSF were normal CPK aldolase and SGOT were normal and LDH slightly elevated but the isoenzyme fractions of LDH were within normal limits EMG was consis ert with myopathy Nerve conduction velocity was normal

A biopsy from the deltoid muscle was fixed in phosphate buffered 4% formaldehyde (pH 7 4) Paraf

[1] Report of the fourth patient has been added in



Fig 7 Electron micrograph demonstrating axial and cross striations of some of the longitudinally cut rod bodies ×26 000

servation by an orthopaedic hospital and at 5 years a transposition of the right posterior tibial muscle into the dorsum of the foot was performed During the last 3 years active physiotherapy has been given and no significant progression has been noted

In November 1970 the patient was admitted to the Children s Hospital University of Helsinki Sh was in good general condition and her mental status was normal She had an elongated expressionless face high palate accentuated lumbar lordosis waddling gait and foot drop Her speech was indistinct and high pitched She had difficulty in opening her eyes fully and she could not lift her head when lying on her back The joints were hyperextensive but there was no significant muscular atrophy Generally he flexors were more affected than the extensors The tendon jerks were absent or very weak and symmetrical Otherwise h r status was normal Blood count urinalysis skull X ray EEG and cerebrospinal fluid were normal CPK aldola e LDH isoenzyme fractions and SGOT were within normal limits The EMG findings recorded in 1966 and 1970 were similar and revealed the changes found in myopathies

A biopsy specimen from the deltoid muscle processed and investigated as in the ca e of the preceding patient revealed an identical morphological picture

DISCUSSION

Our three patients exhibited a fairly uniform clinical picture They were unable to lift their



Fig 8 Connection of rod bodies with expanded Z bands (arrow) ×24 000

heads when lying on their backs owing to weakness of the neck muscles As there was also weakness of the hip and trunk muscles rising from bed was accomplished by a special manoeuvre which was seen in all three children the child first turned on his stomach then put his feet on the floor beside the bed and raised his trunk with the help of his arms This symptom has also been reported by others (4 12 16) However it is not specific and has been described for example in congenital muscular dystrophy and in limb-girdle dystrophy (11 12)

Two of our three patients had breathing difficulties and one of these had them already at birth These respiratory difficulties apparently due to weak respiratory muscles have often resulted in severe pulmonary infections and even death in infants (25) children (16 18) and adults (15) One patient had had two male siblings with muscular weakness similar to that seen in nemaline myopathy who died of a respiratory infection at 9 and 11 months respectively (10) In addition one sister of a patient died at 30 months because of broncho pneumonia The sister was remarkably similar

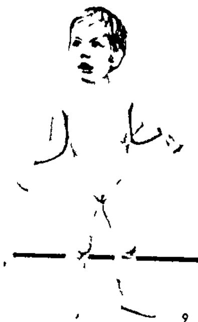






Fig 9 M E patient with nemaline myopathy Notice the myopathic facies

Fig 10 M E The myopathic facies of the same patient

Fig 11 M E Poor support of the head due to weak neck muscles

to the patient both in appearance and in muscular weakness (18)

Though the disease is said to affect the proximal muscles predominantly (9) in two of our patients the weakness of the dorsiflexors of the feet was especially troublesome The literature also contains references to the distal muscular weakness weak ankles at 5 years and difficult to ride bicycle (13) difficulties in running and climbing stairs (3 27) Two of our patients sought medical advice because of nasal voice and indistinct speech which was not caused by the muscular weakness alone This weak nasal, high pitched voice had been noticed in 9 out of 13 patients with early onset of nemaline myopathy (Table 1)

Differential diagnosis in cases of floppy children with none of the symptoms or laboratory findings of the more common neuromuscular disorders is a difficult task Muscle histology supplemented with histochemistry and electron microscopy may be rewarding in this respect (4) Such entities as myotubular myopathy (4 11 12) central core myopathy (4 11 12) megaconial myopathy (4 11 12) pleoconial myopathy (4 11 12) and mitochondrial abnormalities as well as nemaline body disease (4 11 12) are usually diagnosed with the aid of these techniques alone

Table 1 *Clinical data in patients with nemaline myopathy in earlier reported and present cases (1 3 5–8 10 13–27)*

Group	No of cases	Fe males	Median age at diagnosis (years)	Myo-pathic facies	Weak nasal high pitched voice[a]	Normal voice[a]	Sucking disturbed	Weak neck muscles	Respiratory difficulties
I Early onset	25	17/[illegible]3	8 5 (0 17–18)	17/18	9/13	4/13	6/7	8/8	9 9
II Late onset	5	4/5	51 (39–63)	1/2	1/5	0 5	1/3	5 5	[illegible] 4
Present cases	3	2/3	6 2 (3–7 8)	3/3	3/3	0 3	2/3	3/3	2/3

a n/nn number of cases with positive findings/number of cases in which this particular symptom was mentioned in the literature

Although the clinical syndrome in our patients was uniform and consistent with a number of other reports (see Tables 1 and 2) it is not specific A short review of hospital records disclosed 8 patients with early non progressive muscle disorder The nemaline myopathy was excluded as follows the old biopsy slides were reinvestigated and new sections were stained with trichrome and PTAH None of the slides showed nemaline rods We gained the impression that these 8 patients belonged to the group of new myopathies as described by Dubowitz (4) In addition we have recently investigated a 6 year-old girl with muscular weakness from birth and presenting the same clinical picture as our present nemaline body patients Her muscle biopsy however revealed changes indicative of muscular dystrophy with myopathic electromyography and slightly elevated muscle enzymes

Although the previously published children and present cases represent a clinically and pathologically well-delineated disorder it differs from the musculopathies with rods in the few late-onset cases In their patient Heffernan et al (14) found muscle damage suggesting both neurogenic and myogenic origin and a second biopsy from a minimally affected muscle was needed to show rod like

Table 2 *Clinical data in patients with nemaline myopathy in earlier reported and present cases (1 3 5–8 10 13–27)*

Group	No of cases	Deep tendon reflexes		Walking		Course of the disease		High arched palate	Scoliosis lordosis	Pigeon breast or Pectus excavatum	Myopathic electro myo-graphy[a]
		Diminished	Absent	Able to from (years)	Difficulties	Non progr	Slight progr				
I Early onset	25	2/22	15/22	1 8 (norm –4 44)	14/18	16/23	7/23	14/20	6/19	4/ 0	21/24
II Late onset	5	3/5	[illegible]/5	[illegible]	5/5	2/5	3/5	2/2	2/[illegible]	0/2	4/4
Present cases	3	3/3	0 3	1 5 (1–2 5)	3/3	2/3	1/3	3/3	3/3	0/3	3/3

a n/nn number of cases with positive findings/number of cases in which this particular symptom was mentioned in the literature

structures Both the cases with adult onset of Engel et al (6) suffered from other muscle affecting diseases, dermatomyositis and polymyositis, in addition to the nemaline bodies These findings led the latter authors to postulate that rod structures may be unspecific and secondary to the myositis This possibility is corroborated by the findings of Cape et al (2) who observed rod like bodies in the second biopsy of a myositis patient In these cases in which rod formation may be secondary the disorder has been progressive in contrast to the early onset patients

Hopkins et al (15) reported a mother and daughter with nemaline body disorder without any evident primary disease The symptoms of the daughter were observed at the age of 8 years and the clinical picture was nonprogressive and closely resembled that in the other published early onset cases The clinical disorder of the mother first began at the age of 45 years suggesting the possibility that the disorder may manifest late in adult life

The question of whether the nemaline body disease is hereditary cannot yet be solved Some reports (13 20 24) describe affected siblings with healthy parents suggesting autosomal recessive transmission On the other hand in three previous reports (1 15 25) a clinically affected mother and daughter are described Only in the paper of Hopkins et al (15) was the muscle pathology nemaline body disease in both mother and daughter In the large biopsies taken from the deltoid muscles of both parents of our patients No 1 and 3 we could not find any morphological abnormalities in the light microscope or in the electron microscope We think that the early onset myopathy with a large number of nemaline bodies as the only abnormality in the muscle biopsy forms a well delineated nosological entity, although the bodies may sometimes be formed as a result of previous muscle damage of unknown character as is suggested in some of the late onset cases (2, 6)

SUMMARY

Three children with nemaline myopathy a 6-year old boy and two girls aged 3 and 7 years are reported These children have all the characteristics typical of this disorder with early onset namely inability to hold up the head when in a supine position myopathic facies weak nasal high pitched voice diminished deep tendon reflexes waddling gait high arched palate accentuated lumbar lordosis myopathic electromyography Biopsy of the deltoid muscle revealed the typical finding of nemaline bodies, but in both parents of patients 1 and 3 the biopsy finding of the same muscle was completely normal The course of the disease was nonprogressive in all three cases

Note added in proof After submission of this paper for publication we found that the findings in a case previously examined at this hospital were consistent with the picture of nemaline myopathy This girl who is now 11 years old showed muscular weakness with difficulties in sucking and breathing after birth Her mental development has been normal but she did not start walking until the age of 1 7 years She has a severe lordosis and foot drop and there is also weakness and atrophy in the upper extremities in the scapulo-humeral region in particular Her voice is nasal and weak and she has micrognathia and a high and narrow palate In the last few years she has easily become short of breath The muscle enzymes have been within normal limits The EMG recorded several times since 1966 has been interpreted as consistent with myopathic lesions A muscle biopsy specimen taken in 1966 was considered characteristic of muscular dystrophy Reinvestigation of this specimen now revealed accumulations of typical nemaline bodies

ACKNOWLEDGEMENT

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CASE REPORT

PYKNOCYTOTIC HAEMOLYTIC ANAEMIA OF THE NEWBORN

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In 1959, Tuffy et al (16) described 11 infants with haemolytic anaemia associated with pyknotic erythrocytes, for which they used the term infantile pyknocytosis Afterwards, Zannos-Mariolea et al (18) reported 12 cases in 1962, Keimowitz & Desforges (9) one in 1965, Oski et al (11) one in 1965, and Ackerman (1) seven cases in 1969

Pyknocytes are erythrocytes usually smaller than normal, they are distorted contracted and fragmented, with irregular cell borders, often with pointed processes and they stain densely (6, 10, 12 16)

The presence of a small number of pyknocytes seems to be common both in full term and premature infants during the first 3 months of life Tuffy et al (16) found 0 5–1 9% pyknocytes in full term infants aged 5–8 weeks and 0 3–5 6% in prematures aged 1–83 days The number increased within the first 2–3 months of life and fell rapidly after the age of 3 months In adults pyknocytes invariably constitute less than 0 3% However in infantile pyknocytosis the number of these cells was considerable up to 50% The existence of a relationship between pyknocytosis and haemolytic anaemia was suggested by the fact that the number of pyknocytes fell in parallel with the abatement of the haemolysis

As only a few cases of this syndrome are on record, it is as yet not quite clear whether it is a well defined clinical entity It must therefore be of value to report new cases, which is the purpose of this paper

CASE REPORT

In January 1969 a 5 week old boy (CH 121?68) was admitted to our department because of jaundice and anaemia

The patient was the second of two children there was no family history of haemolytic anaemia He was delivered by Caesarean section in a special maternity clinic 3 weeks before term because of total placenta praevia birth weight 2 460 g Menadion 1 mg was given immediately on birth Respiratory insufficiency was present during the first 24 hours Mild jaundice was observed during the third to the eighth day of life At discharge on the 19th day the infant weighed 2 570 g and no jaundice was present at that time The public health nurse observed jaundice when the baby was 4 weeks old

On admission pallor of the skin and mucosae and jaundice were revealed The spleen and liver were not enlarged The patient was not acutely ill

Laboratory examinations Haemoglobin 6 g per 100 ml erythrocytes 1 6 million reticulocytes 28 serum bilirubin 14 9 mg per 100 ml Leucocyte counts showed 12 000 cells per μl with a normal distribution no immature or otherwise abnormal cells Smears showed a markedly pathological red cell morphology with some anisocytosis pronounced poikilocytosis distinct polychromasia a few spherocytes and 13–15 erythroblasts per 100 leucocytes The abnormally formed erythrocytes which stained densely constituted about 20 (Fig 1) The cells were similar to those previously described as pyknocytes (16) Bone marrow from the tibia was hypercellular morphologically normal with increased normoblastic erythropoiesis The erythroblasts constituted 50–60 of the nucleated cells of the bone marrow

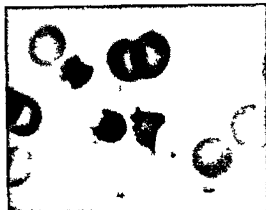


Fig 1 Pyknocytes

The blood groups of the mother and infant were A Rh positive and B Rh positive respectively The direct Coombs test was negative An extensive study for red cell antibodies performed by the Blood Grouping Laboratory Aarhus Kommunehospital did not reveal any antibodies The mother s blood did not contain fo tal erythrocytes The osmotic fragility of the eryth rocytes was normal also after incubation at 38 C for 4 hours The parents and the sister showed nor mal red cell morphology reticulocyte counts and osmotic fragility The glucose 6-phosphate dehydrogenase and pyruvate kinase levels of the red cells were normal The cytomegalo-virus test and toxoplasmosis-complement test were negative and so was the Wassermann reaction There were no signs of Heinz body formation in the erythrocytes. Serum creatinine and serum glutamic pyroracemic acid transaminase were normal The platelets showed no abnormalities

Course of the disease After 10 days in hospital signs of decreasing haemolysis were observed the haemoglobin level began to increase slowly and the jaundice faded After one month the haemoglobin was 9 g per 100 ml and reticulocyte counts showed nor mal values pyknocytes were still present but in a smaller number The haemoglobin level continued to increa e it was 10 5 g per 100 ml at 4 months and 12 0 g at 6 months and at that time blood smears were normal (Fig 2) No blood transfusions had been given At the time of writing, 30 months after the haemolytic episode the boy is still in good health

DISCUSSION

In the case reported above the diagnosis of haemolytic anaemia was supported by the con current jaundice and signs of increased ery thropoiesis (polychromasia reticulocytosis

Fig 2 The number of pyknocytes in the blood during the course of haemolytic anaemia in a newborn infant

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reminiscent of those seen in toxic haemolytic anaemia hepatic or renal insufficiency (15 17) and in micro-angiopathic haemolytic anaemia (3) The vitamin K dose given to the patient on birth was much smaller than those which have given rise to haemolytic anaemia (7 10 12) The time of the occurrence of anaemia weighs against such a causal relationship (2 10) and also against the assumption that the anaesthesia should have been a contributory factor In addition the absence of Heinz bodies also suggested that the disease was not a toxic haemolytic anaemia (2 6)

On the basis of the theory advanced by Brain et al (3) viz that pathological erythrocytes may be produced mechanically by the exposure of the cells to damaged blood vessels Ackerman (1) suggested that a transient defect in the newborn s vascular system could be responsible for the damage to the erythrocytes in pyknocytosis In our case it would be reasonable to consider if the respiratory insufficiency during the first 24 hours of life might have given rise to a vascular lesion Such a vascular theory would offer a satisfactory explanation of (*a*) the self limiting nature of the disease (*b*) the damage to the donor cells and (*c*) the absence of morphological changes in normal red cells which are exposed *in vitro* to sera from newborn infants with pyknocytosis (1)

At present infantile pyknocytosis is thus conceived as a haemolytic anaemia which is characterized by a morphological abnormality in the erythrocytes probably brought about by an extracorpuscular factor Unlike other haemolytic anaemias with abnormal erythrocytes (spherocytosis elliptocytosis stomatocytosis) infantile pyknocytosis is thus apparently not a hereditary defect but a transient abnormality which may be encountered in infants within the first few months of life As the abnormal cells may occur unaccompanied by clinical symptoms the condition is presumably an accentuation of an apparently normally occurring developmental phenomenon (9 12)

SUMMARY

Haemolytic anaemia was diagnosed in a 5 week-old boy The occurrence of pyknocytes in the peripheral blood was a conspicuous feature A correlation between the number of pyknocytes and the severity of the haemolysis was observed The symptoms showed gradual abatement and the red cell morphology had returned to normal when the infant was 6 months old The clinical picture and the course of the disease are characteristic of infantile pyknocytosis

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erythroblastosis and erythropoietic hyperplasia of the bone marrow)

Bleeding as the cause of the increased erythropoiesis could be ruled out Transplacental bleeding combined with physiological jaundice may in certain cases give rise to difficulties in the differential diagnosis in haemolytic anaemia In our case the jaundice developed some time after the physiological jaundice had disappeared and anaemia was not present at the time of birth which excludes such a haemorrhagic anaemia The search for foetal cells in the mother s blood was also negative

It was possible to exclude the varieties of haemolytic anaemia which must generally be considered i e hereditary haemolytic anaemias and erythroblastosis foetalis Thus the self-limiting nature of the disease weighed heavily against the presence of a congenital hereditary erythrocytic defect The results of the laboratory studies ruled out the possibility of hereditary spherocytosis and the most common enzymatic defects The disease could not be referable to Rh erythroblastosis nor to ABO incompatibility as an increased anti B titre was not found in the mother or infant The negative Coombs test excluded auto immune haemolytic anaemia which may occur in all age groups although it is extremely rare in newborn infants There was no clinical or serological evidence suggesting that the condition should be due to some infection Owing to the transient nature of the disease it could be ruled out that it was the type of haemolytic anaemia accompanying acanthocytosis which is thought to be related to a beta lipoproteinaemia (13) and possibly to other factors (8)

It seems reasonable to hold the presence of pyknotic erythrocytes responsible for the haemolysis, as the number of these cells was much larger than that normally found in newborn infants, and also because we like Tuffy et al (16), could demonstrate a correlation between the number of pyknocytes and the severity of the haemolysis during the course of the disease

The case described may thus confirm the assumption that pyknotic haemolytic anaemia is a special syndrome in the newborn In the cases reported in the literature *the clinical picture* has been characterized by jaundice and/or anaemia and occasionally by hepatosplenomegaly (1 16 18) The disease appears within the first few weeks of life more than half of the cases on record began with jaundice within the first few days of life (1 16) However jaundice may not develop until the infant is 3 weeks old (12) the longest duration of this manifestation recorded is 3 weeks (10) Exchange transfusion may be required (1, 16) In five of the 11 patients described by Zannos Mariolea et al (18) signs of kernicterus developed four of the infants died In some cases anaemia does not occur until the age of about one month (12) The haemoglobin level has varied between 4 5 and 10 g per 100 ml After the third month of life the haemoglobin has always shown a distinctly rising tendency In the patients described (except one of Ackerman s children) all signs of haemolysis had disappeared at the age of 6 months and pyknocytes were no longer present at that time (10 12 16) The severity of the haemolysis was correlated to the number of pyknocytes (12 16) The Coombs test has been negative in all cases (1 16) Tuffy et al (16) and Ackerman (1) found an equal sex distribution whereas Zannos Mariolea s group consisted of 10 boys and two girls (18)

The pathogenesis of this form of haemolytic anaemia is completely unknown It seems possible that genetic factors play a certain part in the aetiology (1 16) Some authors have considered a relationship between pyknocytosis and a transient defect in erythrocyte metabolism (4) or an enzyme deficiency (11, 18) However most patients with enzyme deficiencies do not have pyknocytosis (5 12) Investigations on exchange transfusions (1 16) and erythrocyte survival studies (9) have rendered it likely that the haemolysis is due to extracorpuscular factors The morphology of the erythrocytes also supports this assumption because the cellular abnormalities are highly

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CASE REPORT

CYTOSINE ARABINOSIDE IN A CASE OF RETICULUM CELL LEUKEMIA

J ARMATA and J ZAJĄCZKOWSKI

From the Second Paediatric Clinic (Head B Halikowski) Paediatric Institute of the Medical Academy Cracow Poland

Reticulum cell leukemia shows little or no response to various forms of treatment (1 2 5 7) In the patient reported here treatment with cytosine arabinoside proved to be effective in the first blastic phase and in relapses of the disease

CASE REPORT

Anna, a 13 year old girl was admitted to the clinic May 28 1969 because of increasing pallor and weakness during the last few weeks On admission the child was very pale but the lymph nodes liver and spleen were not enlarged Examinations revealed Hb 5 0 g 100 ml r b c 1 760 000/mm w b c 3 600/mm with metamyelocytes 1% juvenile neutrophils 8% segmented neutrophils 53% lymphocytes 36 monocytes 1% and lymphoplasma cells 1% Myelograms performed on May 28 and June 6 showed diminished numbers of granulocytic and erythroblastic cells and a predominance of lymphoid reticulum cells 40–70 of all th cells (Fig 1) Prednisone 3 mg/kg body weight was administered daily Vincristin rubidomycin and nitro en mustard were successively instituted Three months treatment failed to bring about a bone marrow remi sion Numerous blood transfusions were given to ameliorate the toxic effects of the drugs and the anemia

In the beginning of the fourth month of hospitalization the bone marrow was still dominated by lymphoid reticulum cells (66%) which also appeared for the first time in the peripheral blood (280 mm) Then while the prednisone was gradually discontinued the administration of cytosine arabinoside (cytarabine[1]) was begun on Sept 5 1969 by intravenous injection in a single dose of 3 mg/kg body weight daily After 7 injections in the course of 3 weeks the reticulum cells dropped to 4 8% During the remission cytosine arabinoside was continued in a dose of 3 mg/kg every three weeks, and in addition endoxan (5 mg/kg body weight) was given twice a week

On Jan 10 1970 a follow up examination revealed the first relapse of the disease (47 % reticulum cells in the myelogram) Administration of cytosine arabinoside in 5 injections in the course of 2 weeks in a total dose of 15 mg/kg body weight was followed by a drop in the bone marrow parablasts to 4 5% During remission, the girl remained at home but did not take endoxan injections as recommended

The next hospitalization on March 16 1970 was caused by in reasing bone pains and the myelogram showed a relapse with 65% reticulum cells For two weeks she was treated with prednisone (about 1 mg/kg body weight) and L asparaginase (total dose 2 000 u/kg body weight) In spite of this treatment reticulum cells appeared in the peripheral blood (621/mm) Cytosine arabinoside was then reinstituted with a total dose of 36 mg/kg body weight for 17 days A follow up myelogram on April 20 1970 showed remission (3% reticulum cells) As in previous courses of treatment administration of cytosine arabinoside was accompanied by nausea and vomiting neutropenia (more pronounced this time) and thrombocytopenia During remission the patient was treated with cytosine arabino ide in doses of 1 5 mg/kg body weight every 3 weeks and in addition with mercaptopurine methotrexate and endoxan successively

A third relapse occurred on July 30 1970 again accompanied by osteoarticular pains After 8 injections of cytosine arabinoside in the course of 13 days (total dose 36 mg/kg body weight) the number of reticulum cells in the bone marrow dropped from 78 5% to 9% Severe neutropenia and thrombocytopenia made it necessary to discontinue the treatment Pancytopenia associated with the chronic course of the

[1] Supplied by Upjohn Co Kalamazoo USA

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Key words Haemolytic anaemia pyknocytosis

PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting March 10 1971

J Cohn H K Hanel & B Harvald *Adenosine triphosphatase (ATP ase) deficiency in a family with non spherocytic haemolytic anaemia*

This condition was encountered in a boy aged 2¹/ years and it was demonstrated that the patient s mother and maternal grandmother had the same defect but were clinically healthy Two further members of the family were clinically ill viz two of the patient s mother s brothers One of these died at the age of one year and the other underwent splenectomy at the age of five years By means of digoxin inhibition of ATP ase this could be subdivided into two isoenzymes viz. the sensitive ATP ase and the insensitive ATP ase From this it was apparent that the patient had reduced quantities of sensitive ATP ase probably on account of reduced synthesis of the enzyme The mode of heredity is probably irregularly dominant

Discussion

N J Brandt It seems to be difficult to elucidate the mode of inheritance from the information available It appears that there is not always agreement between the enzyme genotype and the phenotype

H K Hanel We are unable to explain why women apparently may have very low serum ATP ase values and yet not exhibit haemolytic anaemia

N J Brandt M Hilden F Schønheyder & F Quaade *A case of homocystinuria*

The first case of homocystinuria diagnosed in Denmark is presented

Case history A woman aged 22 years became pregnant with the same man on five occasions One abortion occurred in the third month of pregnancy and all of the other pregnancies were terminated in approximately the seventh month on account of foetal death Since the first pregnancy the patient had had slight arterial hypertension with a diastolic pressure of about 110 mmHg and occasionally in connection with the deliveries there had been slight increase of the blood pressure (maximum 180/120) and slight proteinuria In the four pregnancies which were terminated the foetuses weighed from 700–1 500 g and measured from 35 to 42 cm long On each occasion a number of white infarcts of 2–4 cm in diameter were observed in the placentas On two occasions autopsy was performed on the foetus but nothing abnormal was demonstrated Following the last delivery which occurred in Maternity Department B Rigshospitalet Copenhagen the eyes of the foetus were submitted to detailed investigation but no pathological changes were demonstrated More detailed assessment of brain tissue did not prove possible on account of maceration

After the fourth delivery the patient was referred in 1970 for further investigation and treatment In October of the same year the

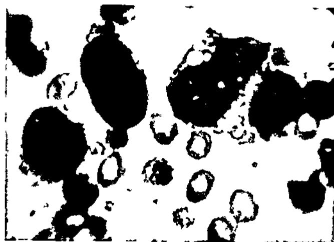


Fig 1 Bone marrow aspirate showing typical reticulum cells (Giemsa stain × 1 000)

disease and or the toxic effects of the drugs prevented further treatment The girl died at home on Febr 1 1971

DISCUSSION

In a case of reticulum cell leukemia refractory to other drugs cytosine arabinoside was administered four times during the blastic phases The first three courses brought about bone marrow remission and the fourth course merely a marked reduction of lymphoid reticulum cells in the bone marrow

Cytosine arabinoside was administered in smaller doses together with other drugs during the first and third remissions which were of short duration

In the entire course of the disease the total dose of the drug was 139 mg/kg body weight Toxic symptoms were nausea and vomiting neutropenia, thrombocytopenia and raised transaminase levels

Cytosine arabinoside has been used in acute lymphoblastic and myeloblastic leukemia in leukemia of the central nervous system and in various malignant diseases (3 4 6) It may also prove useful for inducing remission in some forms of reticulosis

SUMMARY

In a 13-year old girl cytosine arabinoside was administered in four courses during the blastic phases of reticulum cell leukemia resulting in bone marrow remissions after the first three courses Small doses of cytosine arabinoside were administered together with other drugs during the first and third remissions which were of brief duration

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were submitted to the cyanide nitroprusside test In 10 ◦ the reaction was doubtful or typical

These urines were subsequently investigated quantitatively by ion-exchange chromatography (Technicon Auto-Analyzer) Attempts were then made to identify ninhydrin positive substances with the same relative retention time as homocystine This was undertaken partly by means of addition of a standard solution of homocystine to the specimen ("additional peaks) and also by repeated analyses in an altered chromatographic system (different pH and salt gradient)

As a control urine from a patient with homocystinuria was employed (kindly sent by Professor Dent London) As an example patient E K is mentioned She is severely retarded mentally and has excessive myopia cerebral paresis and a characteristic appearance with pronounced malar flush A ninhydrin positive substance was found in the urine which revealed additional peaks in the first system In the second system however the p aks had different retention times We were thus unable to confirm our first presumption that the sub tance was homocystine

We still do not know what is wrong with the patient

Erik Wamberg & Hanne Bjørner *Treatment of phenylketonuria in the Kennedy Institute The results and problems during a three year period of activity*

Since October 1967 35 children (24 boys and 11 girls) with phenylketonuria or hyperphenylalaninaemia were treated by diet in the Kennedy Institute

Eighteen of the cases were discovered by means of the Guthrie test seven by means of Phenistix and the remainder were diagnosed on admission to paediatric departments on account of clinical symptoms

The following were employed as criteria for establishing the diagnosis of phenylketonuria 1) Serum phenylalaninine values over 15 mg% with tendency to increase 2) normal serum tyrosine and 3) demonstration of ketone bodies in the urine

For differential diagnosis from hyperphenylalaninaemia phenylalanine loading was undertaken at the age of 6 months and observation of the phenylalanine tolerance were employed

As the basis of the dietary treatment the phenylalanine free protein hydrolysate Albumaid P[x] was employed and supplemented by milk vegetable purees fruit and starch bread with low phenylalanine content

By means of weekly fasting blood tests a therapeutic serum phenylalanine level of 5–10 mg/100 ml is maintained Outpatient follow up every third to sixth month included investigation of weight height circumference of the head centres of ossification EEG and psychological testing employing Cattell s method

The results of dietary treatment as regards the intellectual development are compared with the time of initiation of treatment for the 25 children Significant difference in the average IQs of children treated from the first month of life (101 3 ± 6 4) and children treated from the second and third months of life (87 1 ± 7 1) was encountered and further emphasizes the necessity of establishing the diagnosis and commencing treatment within the first weeks of life

Various diagnostic and therapeutic problems are mentioned including the question of when dietary treatment may be withdrawn

Discussion

N J Brandt Have supplements with tyrosine and/or glutamine been attempted?

E Wamberg No We have not much faith in such treatment

N J Brandt It appeared from the lecture that treatment commenced after the third month of life has no definite effect Have you the moral courage to undertake a controlled investigation of the effect of treatment in pa-

diagnosis of homocystinuria was established At this time, the patient was four months pregnant and the blood pressure was under relatively good control with diuretics and methyldopa In an attempt to normalize the amino acid metabolism treatment was commenced with 500 mg pyridoxine tablets daily from the end of October and 20 mg folic acid tablets daily from the end of November The patient felt well during the entire pregnancy

The fifth pregnancy was terminated artificially in the middle of December on account of foetal death During the two subsequent months the patient was observed without therapy in any form in order to observe the hypertension and the amino acid metabolism

Objective investigation revealed bilateral lenticular dislocation No cataracts were present and the fundi were normal The palate was high but there were no other symptoms suggestive of Marfan s syndrome The blood pressure was 160/110 mmHg The patient was otherwise found to be quite normal and in particular she was of normal intelligence and with a good social status as an independent bread-winner

The diagnosis was established from 1) a strongly positive cyanide nitroprusside reaction in the urine 2) demonstration of homocystine in the urine by thin layer chromatography and paper chromatography and 3) column chromatographic amino acid determinations in the plasma and urine which revealed great quantities of homocystine and considerably raised methionine values

Homocystinuria is a rare condition which is inherited by autosomal recessive heredity The disease is due to absence of the enzyme cystathionine synthase which results in increase of methionine in the plasma and urine and considerable homocystinuria and further defective synthesis of cystathionine and defective synthesis of cystine

In typical cases the patients exhibit a clinical picture resembling Marfan s syndrome including lenticular dislocation and in addition mental retardation occurs in half of the cases and thromboembolic episodes osteoporosis and neurological disturbances may be present in a number of cases

(To be published in Ugeskrift for Læger)

Discussion

H K Hanel How does pyridoxine work?

N J Brandt This is not known with certainty Pyridoxine is a co-enzyme for cystathionin synthesis and a few authors have found that the activity of the enzyme in liver tissue increases in patients receiving pyridoxine therapy from approximately 1% of normal to approximately 2% or from 2% to 4%, i e 100% increase in activity which should suffice to explain the normalization of the biochemical parameters Other authors have not been able to confirm increase in activity such as this during pyridoxine therapy

E Wamberg We have encountered several apparent clinical cases but have not been able to obtain biochemical confirmation Are there fewer cases in Denmark than elsewhere?

N J Brandt The disease appears to be most common in Ireland and among Irish emigrants to USA One of the problems may be sending of the specimens of urine These must be fresh since homocystine is readily oxidized Finally the cyanide nitroprusside reaction is occasionally negative in patients so that a single negative test is not conclusive In particularly suspect cases the methionine tolerance test may be of value

A Dupont & F Rosleff We attempted to find patients with homocystinuria in the amino-acid laboratory in Brejning We were particularly interested in mentally retarded patients with clinical pictures resembling Marfan s syndrome and patients with pictures resembling Marfan s syndrome and patients with lenticular dislocation Further the urines of appro[illegible] mentally retarded patients

were submitted to the cyanide nitroprusside test In 10 % the reaction was doubtful or typical

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E Wamberg It is correct that the diagnosis should be established within the third month of life in order to be certain of therapeutic effect On the other hand, it is known that treatment commenced after the third year of life is without significance Nothing definite can be stated about the intervening period A controlled investigation would be desirable but could scarcely be conducted in practice

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As published previously analyses have been undertaken in Denmark of the amino acid content of the phenylalanine free amino acid preparation Albumaid XP® These revealed a relative deficiency of the essential amino acids methionine isoleucine and leucine As a result of this preparation was enriched with the amino acids concerned in an attempt to correct these deficiencies and in this manner an approximation to the pattern of essential amino acids in egg protein was obtained

It has not proved possible to document any decisive differences The reason for this may be that the ordinary standards for amino acid supply are considerably higher than the minimal requirements by means of clinical employment of the ordinary preparation as manufactured and the enriched product in children with phenylketonuria

Nevertheless determinations of the biological value of the preparation compared with egg protein in experiments with young rats during the period of growth reveal that enrichment involves improvement of the biological value from approximately 40% to over 80%

The biological values of other phenylalanine free dietary preparations or preparations with low phenylalanine content, Lofenalac® (enzyme hydrolysate) Cymogran® (acid hydrolysate) and Aminogran® (mixture of purified amino acids) were found to be about 70-75%

By supplementing Albumaid XP® with essential amino acids it has thus proved possible to raise the originally low biological value to higher levels than in the other preparations available It has been agreed that until further notice the factory will undertake enrichment of the preparation exported to Denmark

Discussion

Bent Friis Hansen In conditions of protein deficiency alterations in the amino acid levels are encountered Have quantitative determinations of amino acid in patients with the various dietary preparations been undertaken?

E Olesen & E Wamberg Such determinations are expensive and difficult to carry out but are also desirable An investigation of this nature has been planned in cooperation with *Brenning*

Meeting April 14 1971

Susanne Boelskov N J Brandt & J Cohn *Osteodysplasia with thrombocytopenia*

Our proband is a female infant now aged 9 months with bilateral radial aplasia and amegacaryocytic thrombocytopenia

Delivery was normal and occurred at term following an uncomplicated pregnancy Bi... weight 2 480 g length 46 cm The patient was hospitalized immediately after birth on account of the low birth weight and the malformations These comprise bilateral radial aplasia medial dislocation of the hands and klinodactyly of the fifth finger In addition the blood picture was abnormal constan...

thrombocytopenia (3 000–40 000 per μl) moderate leucocytosis with 5–10% immature leucocytes in the peripheral blood and low haemoglobin percentage In the bone marrow very few megakaryocytes and no thrombocytes were observed

A female cousin of the mother had borne four children the second of whom had the same malformations of the upper limbs but normal haematological status The fourth child died at the age of 4 months from cerebral thrombosis He had the same malformation of the upper limbs and amegakaryocytic thrombocytopenia

Congenital hypoplastic thrombocytopenia with one or more malformations has been described in approximately 40 cases A few of these patients were related to one another The thrombocytopenia is resistant to steroid therapy and splenectomy A few spontaneous remissions have been observed but the prognosis is as a rule very poor The children usually die during the first year of life from cerebral or other haemorrhages

Discussion

J C Melchior Have the genetic conditions been elucidated? How great is the risk that the next child will have the condition?

N J Brandt Several different genetic defects are possibly involved The genetic conditions in this family are not yet elucidated but judging from the literature an autosomal recessive defect may be concerned and the risk that the next child has the disease is thus 25

J Christoffersen *Muscular dystrophy A review*

Muscular dystrophy is defined as a genetically conditioned degenerative myopathy The genuine dystrophies not including the congenital myopathies and the myotonic syndromes may be subdivided thus (J N Walton & D Gardner Medwin 1968)

X linked Severe type = Duchenne Benign type = Becker

Autosomal recessive Duchenne like Limb-girdle

Autosomal dominant Facio-scapulo-humeral Distal dystrophy Ocular dystrophy Oculo-pharyngeal dystrophy

The morphological changes in the dystrophic muscles consist of parallel degenerative and regenerative processes This is reflected also in the biochemical changes which develop foetal traits possibly as a result of relative increase of young cells The primary metabolic defect has not hitherto been elucidated but it has been postulated that there is a neurogenic factor in the pathogenesis

Both the myocardium and smooth musculature may be the site of dystrophic changes and cardio-pulmonary insufficiency is the cause of death in the majority of the patients suffering from the Duchenne type Severe cardiac symptoms may also develop even in the more benign types

Approximately 30% of the patients with dystrophies of Duchenne type have intelligence quotients of under 75 The intelligence defect is genetically associated with the dystrophy and there appears to be complete concordance as regards the intelligence level in the dystrophic members in a particular family The mental reduction does not progress and there is no relationship between the degree of severity of the muscular lesion and the degree of intelligence defect

Known carriers of Duchenne s type of dystrophy may have slight or moderate clinical symptoms and approximately 70 % have raised CFK values in the serum By means of electromyography and muscle biopsy a further 10–20 of the carriers may be revealed Only 50% of the carriers of Becker s type can be demonstrated by this method and in the autosomal recessive types the carriers cannot be revealed

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E Wamberg It is correct that the diagnosis should be established within the third month of life in order to be certain of therapeutic effect On the other hand, it is known that treatment commenced after the third year of life is without significance Nothing definite can be stated about the intervening period A controlled investigation would be desirable but could scarcely be conducted in practice

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As published previously analyses have been undertaken in Denmark of the amino acid content of the phenylalanine free amino acid preparation Albumaid XP® These revealed a relative deficiency of the essential amino acids methionine isoleucine and leucine As a result of this preparation was 'enriched' with the amino acids concerned in an attempt to correct these deficiencies and in this manner an approximation to the pattern of essential amino acids in egg protein was obtained

It has not proved possible to document any decisive differences The reason for this may be that the ordinary standards for amino acid supply are considerably higher than the minimal requirements by means of clinical employment of the ordinary preparation as manufactured and the enriched product in children with phenylketonuria

Nevertheless determinations of the biological value of the preparation compared with egg protein in experiments with young rats during the period of growth reveal that enrichment involves improvement of the biological value from approximately 40% to over 80%

The biological values of other phenylalanine free dietary preparations or preparations with low phenylalanine content Lofenalac® (enzyme hydrolysate) Cymogran® (acid hydrolysate) and Aminogran® (mixture of purified amino acids) were found to be about 70-75%

By supplementing Albumaid XP® with essential amino acids it has thus proved possible to raise the originally low biological value to higher levels than in the other preparations available It has been agreed that, until further notice the factory will undertake enrichment of the preparation exported to Denmark

Discussion

Bent Friis Hansen In conditions of protein deficiency alterations in the amino acid levels are encountered Have quantitative determinations of amino acid in patients with the various dietary preparations been undertaken?

E Olesen & E Wamberg Such determinations are expensive and difficult to carry out but are also desirable An investigation of this nature has been planned in cooperation with Brejning

Meeting April 14 1971

Susanne Boelskov N J Brandt & J Cohn *Osteodysplasia with thrombocytopenia*

Our proband is a female infant now aged 9 months with bilateral radial aplasia and amegacaryocytic thrombocytopenia

Delivery was normal and occurred at term following an uncomplicated pregnancy Birth weight 2480 g length 46 cm The patient was hospitalized immediately after birth on account of the low birth weight and the malformations These comprise bilateral radial aplasia medial dislocation of the hands and klinodactyly of the fifth finger In addition the blood picture was abnormal constant

thrombocytopenia (3 000–40 000 per μl) moderate leucocytosis with 5–10% immature leucocytes in the peripheral blood and low haemoglobin percentage In the bone marrow very few megakaryocytes and no thrombocytes were observed

A female cousin of the mother had borne four children the second of whom had the same malformations of the upper limbs but normal haematological status The fourth child died at the age of 4 months from cerebral thrombosis He had the same malformation of the upper limbs and amegakaryocytic thrombocytopenia

Congenital hypoplastic thrombocytopenia with one or more malformations has been described in approximately 40 cases A few of these patients were related to one another The thrombocytopenia is resistant to steroid therapy and splenectomy A few spontaneous remissions have been observed but the prognosis is as a rule very poor The children usually die during the first year of life from cerebral or other haemorrhages

Discussion

J C Melchior Have the genetic conditions been elucidated? How great is the risk that the next child will have the condition?

N J Brandt Several different genetic defects are possibly involved The genetic conditions in this family are not yet elucidated but judging from the literature an autosomal recessive defect may be concerned and the risk that the next child has the disease is thus 25 %

J Christoffersen *Muscular dystrophy A review*

Muscular dystrophy is defined as a genetically conditioned degenerative myopathy The genuine dystrophies not including the congenital myopathies and the myotonic syndromes may be subdivided thus (J N Walton & D Gardner Medwin 1968)

X linked Severe type = Duchenne Benign type = Becker

Autosomal recessive Duchenne like Limb-girdle

Autosomal dominant Facio-scapulo-humeral Distal dystrophy Ocular dystrophy Oculo-pharyngeal dystrophy

The morphological changes in the dystrophic muscles consist of parallel degenerative and regenerative processes This is reflected also in the biochemical changes which develop foetal traits possibly as a result of relative increase of young cells The primary metabolic defect has not hitherto been elucidated but it has been postulated that there is a neurogenic factor in the pathogenesis

Both the myocardium and smooth musculature may be the site of dystrophic changes and cardio-pulmonary insufficiency is the cause of death in the majority of the patients suffering from the Duchenne type Severe cardiac symptoms may also develop even in the more benign types

Approximately 30% of the patients with dystrophies of Duchenne type have intelligence quotients of under 75 The intelligence defect is genetically associated with the dystrophy and there appears to be complete concordance as regards the intelligence level in the dystrophic members in a particular family The mental reduction does not progress and there is no relationship between the degree of severity of the muscular lesion and the degree of intelligence defect

Known carriers of Duchenne s type of dystrophy may have slight or moderate clinical symptoms and approximately 70% have raised CFK values in the serum By means of electromyography and muscle biopsy a further 10–20% of the carriers may be revealed Only 50% of the carriers of Becker s type can be demonstrated by this method and in the autosomal recessive types the carriers cannot be revealed

J Christoffersen & A Leth *Neuromuscular diseases in children A material from 11 years in Department G Rigshospitalet Copenhagen* The case histories of 89 children with primary neuromuscular diseases were reviewed regarding the values of the current diagnostic tests 45 children had muscular dystrophy 19 had spinal muscular atrophy, 9 benign hypotonia 3 polymyositis and 6 had other conditions with muscular symptoms including myositis ossificans polyneuropathy and Thomsens myotonia congenita

The clinical symptoms of crawling up themselves and pseudohypertrophy of the muscles of the calves were described in the majority of patients with muscular dystrophy regardless of the type but were also found in occasional patients in the remaining groups

Muscular biopsy and EMG did not invariably reveal changes which agreed with the diagnosis and in each of the main groups there were occasional patients in whom the results of one or more tests were characteristic for the other group EMG revealed however the greatest diagnostic certainty Out of the patients with muscular dystrophy in whom both biopsy and EMG had been undertaken approximately $^{1}/_{4}$ had characteristic changes in the EMG alone while approximately $^{1}/_{8}$ had characteristic changes in the biopsy findings alone In patients with spinal muscular atrophy considerably better agreement was found It was apparent from the material that the site and the time of testing in relationship to the duration of the condition were of decisive significance for the demonstration of changes

Muscular enzyme investigations revealed characteristic changes in five out of seven patients with muscular dystrophy

The levels of the serum enzymes were registered and where CFK is concerned this was found to exceed the normal values by several hundred per cent in 8 out of 12 patients with Duchenne type and Duchenne like dystrophies while the values were only slightly raised in the remaining four patients Three patients with other types of dystrophy had normal CFK values at the time of investigation Moderate increases in CFK values were also encountered, however, in 4 out of 8 patients with spinal muscular atrophy and in one patient with polyneuropathy while two patients with polymyositis had normal CFK values GO transaminase and total LDH were similarly found to be raised not only in patients with myogenic conditions but also in patients with neurogenic conditions while GP transaminase was found to be raised only in the patients with muscular dystrophy (22 out of 36) and in one patient with polymyositis Investigation of LDH isoenzymes in the serum revealed a relative increase of the first three fractions in 9 out of 13 patients with muscular dystrophy but the same pattern was encountered in 4 out of 8 patients with spinal muscular atrophy On the whole the serum enzyme determinations could thus render an impression of progressive degeneration of muscular cells but with the exception of CFK the differential diagnostic value of serum enzymes appeared to be limited

Attention is drawn to the fact that transient ischaemia of an extremity may result in marked increase in CFK in the serum in patients with muscular dystrophy whereas in normal individuals under similar circumstances a fall in CFK occurs

The urinary creatinine index was found to be abnormal in all of the patients investigated in the material and comparison of the excretory conditions in patients with dystrophies and atrophies did not reveal definite differences This investigation is therefore of doubtful value in the differential diagnosis

Investigation of urinary amino acids revealed abnormal conditions in occasional cases only and no characteristic features in the amino acid pattern were found in the individual disease categories

Discussion

Sven Brandt Were the negative EMGs from small children?

J Christoffersen No the negative EMGs were mainly those undertaken earlier in the investigation Perhaps this is due to altered technique

O Thoge No the technique and interpretation have not altered since the first description was made Was affected muscle always submitted to investigation?

J Christoffersen Yes

O Thoge In my opinion the muscle biopsies should be reviewed in the patients with negative EMGs I consider that enzyme histochemistry will prove of considerable diagnostic value in future

J C Melchior I consider that it is satisfactory that such good agreement has been found between biopsy EMG and enzyme determinations in this material

H Dyggve How many of the hypotonic patients recovered?

J Christoffersen A follow up investigation of the benign hypotonias is in progress

P Plum One sequel of benign hypotonia is oligophrenia A greater proportion than I had imagined become mentally retarded some develop behaviour disturbances while others recover completely

Sven Brandt Is there any therapeutic progress in the progressive muscular dystrophies? Should these patients have physiotherapy? Some authorities have stated that physiotherapy may cause deterioration

J Christoffersen According to the literature physiotherapy should be administered Death is due to cardiac complications

P Plum The ultimate prognosis in progressive muscular dystrophy is hopeless but the patients must be kept moving and attempts must be made to counteract development of contractures Whether physiotherapy can alter the spontaneous course or not is impossible to assess It is important to know when to seek the advice of an orthopaedic surgeon

J Reimers If the sum of the flexion contractures in the knee and hip joint exceeds 60° walking is impossible American orthopaedic surgeons consider that patients can be kept moving until they die

J Leth *Cardiac changes in muscular dystrophy*

A follow up investigation was undertaken on 23 patients with progressive muscular dystrophy which had been verified by laboratory tests clinically and histologically Eleven patients all of whom were boys fulfilled the criteria described by Walton for classification as X linked type while 12 patients three girls and nine boys had Duchenne like muscular dystrophy without familial predisposition The patients submitted to follow up examination had electively illness of long duration and severe motor handicap The average age was 12 3 years (9–18 years)

The ECG changes most frequently encountered were high R waves which were not increased in width in V_{1-} (15 patients) and pathological R/S relationships in V_1 (16 patients) Further deep Q waves which were not increased in width were found in V_{5-6} (6 patients) increase in width of the QRS complex (12 patients) right sided bundle branch block (7 patients) and right sided axis deviation (4 patients) P Q S-T and Q T were normal in all patients A characteristic finding was increased heart rate for the patient s age This was demonstrated in a total of 19 out of 23 patients

None of the 23 patients had entirely normal ECG recordings

The ECG changes which were encountered were all non specific and are most readily explained as part of a disturbance in intraventricular conduction This is in agreement with the diffuse fibrosis frequently demonstrable at autopsy The observation that the

right-sided changes predominate is due to the fact that the right bundle branch is more vulnerable on account of its longer course

The ECG changes which occur most frequently are compared to not only the duration of the disease but also to the degree of motor handicap In both instances increasing incidence of pathological ECG findings (high R in V_{1-}, abnormal R/S in V_1 increased heart rate) the longer the duration of the disease and the more severe the motor handicap

The ECG findings were of no value in the differential diagnosis between the Duchenne and Duchenne like muscle dystrophies as the number of pathological ECG findings were identical in the two groups of patients

Ordinary clinical investigation revealed slight right sided cardiac decompensation in one case only, while no cases of left sided cardiac decompensation were encountered In all of the cases, the cardiac volume was found to be within normal values and the blood pressure was normal in all of the patients with one exception Stethoscopic examination revealed systolic murmurs localized to the second left intercostal space in a total of four cases The murmurs could not be characterized as definitely pathological in any of the cases

Thus, a cardiac component of the muscular condition could only be demonstrated by ordinary clinical investigation in one case, while the ECG recordings were abnormal in all cases

It is concluded that the ECG is an important diagnostic investigation which should be undertaken routinely in all patients with muscular dystrophy

Discussion

Sven Brandt Can cardiac involvement be due to the long period of rest in bed?

A Leth Patients do not develop cardiac changes such as these following prolonged rest in bed

Stig Sparrevohn What is the incidence of the disease?

A Leth There are at present about two hundred patients in Denmark

O Thage If the cases of myasthenia and myotonies are included there are approximately 1 000 patients in Denmark with severe muscular conditions

N J Brandt

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting April 30 1971

E Lykkegard Nielsen *Cystic fibrosis Incidence in Denmark*

Previous investigators have found the incidence of cystic fibrosis (CF) to be between 1 1385 and 1 4142 live births In Sweden however Selander found an incidence of only 1 7700 The majority of research workers consider now that the genuine incidence in the white race is between 1 1000 and 1 2000

An attempt was made to trace all cases of CF in Denmark born during the period 1945–1969 by means of questionnaires sent to 278 hospital departments and centres for tuberculosis in this country A total of 301 patients were found of whom 123 still survived at the time of the investigation while 178 had died Further 150 possible cases have not yet been investigated Five patients aged 20–23 years and two aged 19 years are still alive

The number of patients who died increased at first during the period in question and thereafter decreased while the number of surviving patients increased steadily The number of cases of CF diagnosed annually in Denmark increased during the period

A steadily increasing incidence of the disease throughout the period of investigation was found from 1 20 206 to 1 4 530 live births for the entire country The incidence is greater for Copenhagen than for the remainder of the country The apparent increasing incidence of the disease is attributed to improved diagnosis and similarly the apparent greater incidence in Greater Copenhagen is probably due to greater incidence of diagnosis here The greatest incidence was found in infants born in Greater Copenhagen in 1958–1962 viz 1 2 893 live births but even this figure is probably less than the genuine incidence The figure reveals however that the incidence in D nmark is parallel to that in other countries in Europe and USA The incidence found for infants born in the entire country in 1963–1967 of 1 4 530 live births is undoubtedly less than the actual incidence and shows that the diagnosis of CF is still established too seldom in Denmark

E Winge Flensborg *Sweat testing in cystic fibrosis*

In this department we employed the plate method until 1963 Since then the method has been abandoned on account of the excessive number of false positive and false negative results

Until 1964 Mauer & West s method (intradermal metacholine bromide) was employed as a standard method A material of 231 normal individuals was investigated with this method In childhood the upper normal limit for sodium is 60 mEq/l The upper limit for sodium/potassium ratio increases in childhood from approximately 3 to over 4 The upper limit for adults is difficult to ascertain This method is reliable but was abandoned on account of side-effects

A total of 780 tests on 402 normal individuals were undertaken up to 1969 employing the pilocarpine iontophoresis method (Gibson

& Cooke) This method is reliable when it is carried out regularly by one or a few technicians who are responsible for the entire procedure The upper normal limit for sodium from one month to one year was found to be 50 mEq/l and, in the remainder of childhood 60 mEq/l It is difficult to establish the upper limit where adults are concerned

A total of 224 tests in 58 children with CF revealed sodium values which were constantly above the normal limits recorded in 56 of the cases In the two remaining patients the sodium values varied between normal and raised values Normal sweat test results do not exclude CF

Until 1969 this method was employed to screen 882 children with chronic respiratory disease and/or dyspepsia In this manner 20 cases of CF were revealed

Until it is possible to carry out systematic screening in infancy it is recommended that large groups of children with chronic and recurrent respiratory conditions and dyspepsia be screened

The pilocarpin iontophoresis method (Gibson & Cooke) is unsuited for screening as it is very time consuming Suitable rapid screening methods will be mentioned in the next communication

Janne Steinrud *Cystic fibrosis Screening*

Two different electrodes were employed for measurement of the chloride content of the sweat after preliminary pilocarpine iontophoresis

Employing ORION 401 chloride electrode 55 patients with cystic fibrosis were investigated with a total of 80 double tests and 536 individuals without CF with 541 double tests In one of the double tests the measurements were undertaken directly on the skin while in the other, the sweat was absorbed by ash free filter paper and measurements undertaken on this No definite difference was observed between the two methods Employing this apparatus the upper normal limit for chloride was found to be 75 mEq/l (except in the neonatal period) with however slight overlapping in the period one month to one year

Using ORION 417 chloride electrode a total of 71 tests were undertaken in 55 children with CF and 754 tests were undertaken in children who did not suffer from CF With this apparatus the upper normal limit for chloride was 40 mEq/l (except in the neonatal period)

This apparatus is well suited for screening after the age of one month and is practical as the apparatus for iontophoresis and the chloride ionometer are combined In addition the negative and positive electrode for iontophoresis are combined in one unit and disposable pads are employed for both the negative and positive electrodes This method is rapid (approx 6–7 min)

E Winge Flensborg & Janne Steinrud *Electrolytes in the sweat of normal neonates*

By means of the pilocarpine iontophoresis sweat test (Gibson & Cooke) an investigation was conducted on 248 normal neonates (with birth weights ⩾2 500 g) aged from one hour to 30 days a total of 681 tests being undertaken In each infant from one to four tests were undertaken (on different days) Only tests with a quantity of sweat ⩾30 mg (⩾6 mg/ccm skin area) were employed for assessment

The average sodium concentration in the sweat increases from 41 mEq/l in the first six hours of life to between 51 and 55 during the subsequent four days Thereafter the value decreases steadily and the end of the first month of life it attains a level similar to the average value for the first year of life Sweat sodium values of up to 85 mEq/l were observed during the first six days of life

Ther average potassium concentration increases gradually from 12 mEq/l at birth to 19 5 in the third to fourth weeks of life

The average sodium/potassium ratio has

the same pattern as the sodium values viz it increases from 3 7 to 4 5 in the first days of life and this high level (4 3–4 5) is maintained in the second 24 hours after which there is a gradual fall in the course of the first month of life to approximately 1 6

By means of investigations of the chloride concentration in sweat in neonates under taken at a later date this raised electrolyte level was also confirmed Sweat chloride de terminations were undertaken with an elec trode (ORION 417) following preliminary iontophoresis on the skin of 320 neonates (aged 1–30 days) A total of 728 tests were performed

The sweat chloride level is distinctly higher than in the remainder of childhood During the first day of life the average value for Cl rises from 47 to 51 mEq/l and thereafter falls steadily during the first month of life to 26 Cl values as high as 110 mEq/l were encountered during the first four days of life

Definite increase in the concentrations of sodium and chloride in the sweat of normal neonates have thus been demonstrated This holds also true for the Na/K ratio The ap pearance of the graph of the average Na/K ratio during the first month of life corre sponds closely to the form of the graph for the involution of the foetal suprarenal cortex and is reciprocal to the development of the per manent suprarenal gland

The electrolyte pattern demonstrated in the sweat of normal infants during the first month of life is perhaps conditioned endo crinologically and this may contribute to understanding of the altered sweat electrolyte pattern in patients with cystic fibrosis

Discussion

J Vesterdal Is duodenal intubation out of date?

E Winge Flensborg The diagnosis of cystic fibrosis is never established without investiga tion of the pancreatic secretion

C Hansted Is cystic fibrosis never encount ered in patients with normal results of the sweat test?

E Winge Flensborg Yes occasionally but 99% have pathological values

N J Brandt Is the concentration of electro- lytes in the pancreatic secretion abnormal?

E Winge Flensborg We have not investigated this

E Thamdrup Is the copper content of sweat significant?

E Winge Flensborg The copper content is lowered Determinations of this parameter will possibly provide a sharper limit for nor mal values

E Winge Flensborg Klaus Jensen & E Lyk kegaard Nielsen *Intensive treatment of cystic fibrosis*

The pulmonary condition in all patients with cystic fibrosis regardless of whether pulmo- nary complications have appeared or not should be treated intensively with a night mist telt (preferably with a supersonic atom izer) intermittent inhalation therapy (one or more of the following preparations muco- myst carbamide methaoxedrine isoprenaline various antibiotics) pulmonary physiotherapy and postural drainage antibiotics with reg ular bacteriological control (particular anti biotics for staphylococcus aureus and pseudo monas aeruginosa which are the most fre quent bacteria in the lungs) breathing exer cises (older children) physical activity expec torant cough mixtures and in selected cases anabolic steroids The technique of investiga tion of the expectorate or secretion obtained by suction from the respiratory passages is reviewed The specimens should be examined microscopically and culture must be per formed

Infection with staphylococcus aureus can

in the majority of cases be treated effectively with various combinations of penicillin fucidin methicillin and oxacillin and methicillin as inhalants but frequent courses of treatment (and follow up examination) are necessary We have never seen methicillin resistent staphylococci

Infection with pseudomonas is more difficult to treat We employ various combinations of colimycin, carbenicillin oxytetracyclin gentamycin and bactrim and colimycin as inhalant The bacteria are only rarely eradicated but infections are frequently less massive and the patients usually improve clinically The pseudomonas are practically always of a special capsulated type For inhalation and mist tent therapy sterilized water should always be employed as pseudomonas may be encountered in water cleaned by other methods

Frequent use of otological assistance is necessary for treatment of infection of the upper respiratory passages and removal of nasal polypi which frequently recur

Alimentary symptoms are treated with a diet with a low fat content or normal diet with pancreatic enzyme preparations and supplementary vitamins If rectal prolapse has occurred it usually disappears spontaneously during this form of treatment Meconium ileus equivalent can in mild cases be treated with mucomyst orally and enemata containing pancreatin and/or mucomyst

Discussion

E Thamdrup Should abortive cases be treated also?

E Winge Flensborg We dare not withhold treatment in any cases Respiratory function tests demonstrate that it is necessary

Kaare Sloth *Liver and biliary changes in cystic fibrosis* (CF)

Post mortem examination of patients with CF reveals particularly frequently proliferating dilated biliary passages in the liver with precipitation of eosinophile material and accompanied by connective tissue proliferation (focal biliary fibrosis) In rare cases marked fibrosis may occur cutting off regions of the parenchyma which may resemble genuine foci of regeneration or may be of more normal structure (multilobular biliary cirrhosis) The gall bladder is frequently shrunken and possibly with stenosis or obstruction of the cystic duct

Histologically submucous cystic and mucous metaplasia of the epithelium without evidence of infection are found in the gall bladder

Clinically hepato splenomegaly and evidence of portal hypertension may be encountered but as a rule the hepatic changes are symptomless In a number of patients the biliary passages are greatly reduced in size on radiographic examination, the so called micro gall bladder

By reviewing 81 cases histories from patients with CF aged from nine days to 23 years (average 8 1/ years) we found a case of cirrhosis of the liver with portal hypertension and oesophageal varices in a girl aged 17 years In two further patients cirrhosis was suspected In 30 patients radiographic examination of the biliary passages had been undertaken Four of these had micro gall bladders and in nine the gall bladder was not demonstrated and one (or possibly two) had gall stones Values for SGOT alkaline phosphatases prothrombin and serum electrophoresis were frequently pathological but without any definite pattern suggestive of hepatic disease The serum bilirubin and bromsulphalein retension were practically always normal

E Winge Flensborg *Investigation of the effects of testosterone dehydroepiandrosterone and dianabol on the electrolytes in the sweat in children with cystic fibrosis*

The electrolytes in the sweat were investigated before and after treatment with testosterone dehydroepiandrosterone and dianabol in 18

children with cystic fibrosis Six children were investigated with each of the preparations Prior to treatment an average of six pilocar pine iontophoresis sweat tests (Gibson & Cooke) were undertaken in each of the chil dren and during treatment an average of 15 sweat tests were undertaken A total of 381 sweat tests were undertaken

Treatment with testosterone did not result in any change in Na K or the Na/K ratio

Treatment with dehydroepiandrosterone did not have any definite effect on the electro lytes in the sweat either

On treatment with dianabol a distinct fall in the sweat sodium values occurred on an average from 117 to 95 mEq/l This was how ever followed by an increase to the original values where five of the children were con cerned

The Na/K ratio showed an average fall from approximately 8 to 5 5 and this fall per sisted during the period (approximately five weeks) during which the patients were ob- served with the sweat test during dianabol therapy

Dianabol does *not* normalize the electrolytes in the sweat in children with cystic fibrosis but causes a primary fall both of Na and Na/K ratio The prolonged lowering of the Na/K ratio is mainly caused by an increase in the potassium values

The investigation demonstrates however that the electrolytes in the sweat in children with cystic fibrosis may be influenced by a preparation which by means of its relation ship to androgens is of quite a different na ture than the mineral corticoids hitherto known

E Winge Flensborg *The possibility of a spe cific androgen defect in cystic fibrosis*

It appears from the results of research in the past few years that cystic fibrosis (CF) must be considered to be a hereditary inborn error of metabolism

The important discovery of male sterility in CF on account of aplasia of derivatives from the Wolffian ducts make it appear prob- able that male foetuses with CF are deficient either partially or completely in the third to fourth months of intrauterine life in the tes ticular factor (Jost) which determines the de velopment of the Wolffian ducts The nature of this factor (hormone?) is not yet known

Other features support the theory of a spe cial androgen defect in patients with CF de layed development of puberty in boys with CF and retarded development of the centres of ossification most pronounced in girls Further it has been demonstrated that girls with CF of all ages have an excess mortality which is very pronounced after puberty

The high levels of Na Cl and the Na/K ratio in sweat in CF patients and in some nor mal neonates cannot be explained by our pres- ent knowledge of mineral corticoids It is pos sible that normal neonates may have a tran sient deficiency of a factor (an unknown min eral corticoid?) which as in CF patients can determine the constantly high electrolyte lev els in the sweat

In recent years we have worked from the hypothesis that CF patients might be deficient in a factor with certain androgenic and min eral corticoid effects and we have attempted to elucidate this further Some of the results are presented in the subsequent communica tions

Discussion

Svend G Johnsen A severe androgenic de fect in foetal life should result in hypospadias and other serious defects

Erik Thamdrup Delayed puberty may be due solely to the serious illness

Svend G Johnsen How many of the boys with cystic fibrosis lack these sperm ducts?

E Winge Flensborg Nearly 100

P Olavsgaard A M Worm, Else Andersen & E W Flensborg *Development of the centres of ossification in children with cystic fibrosis*

In 58 patients (32 boys and 26 girls) with cystic fibrosis the left hand was x rayed and the bone age determined from Greulich and Pyles atlas and compared with a Danish normal material In this manner we have obtained an expression of the degree of development which is better than e g the chronological age, height and weight

The patient material was very heterogeneous because treatment had been undertaken in various places No form of androgen therapy had however been administered

When the skeletal age and the chronological age are plotted graphically 73% of the girls and 53% of the boys fall below the normal scatter The observation that more of the girls do so is in agreement with the fact that cystic fibrosis is a more serious disease in them and with a greater excess mortality On subdividing the patients into degrees of severity from 1–4 (as estimated particularly by the pulmonary changes) it is shown that in boys there is good agreement between severity of the disease and lowest bone age whereas no definite connection could be demonstrated in girls

By comparing the chronological age of the CF patients with 1) the height and 2) weight in relation to a normal material it was found as anticipated that the height and particularly the weight were definitely reduced and as with the skeletal age this was most pronounced in girls When the height is considered in relation to the bone age the height is found to be relatively more reduced

Thus, children with cystic fibrosis have apparently retarded skeletal development height and in particular, weight These values may therefore be employed to assess the response to therapy

Discussion

Henning Andersen The growth in height is more influenced than the skeletal development which does not support the theory of a specific androgenic defect

Erik Thomdrup The pathological conditions described here are similar to those in other chronic diseases

E Winge Flensborg & Svend G Johnsen *Significant changes in two 17 ketosteroid fractions in cystic fibrosis*

A new method of investigating the 17 ketosteroids in the urine (Girad purifying and gas chromatographic analysis) permits isolation and determination with great accuracy of seven isolated pure 17 ketosteroids androsterone (A) ethiocholanolone (E) dehydroepiandrosterone (DHA) 11 keto-androsterone (KA) 11 keto ethiocholanolone (KE) 11 hydroxy androsterone (OHA) and 11 hydroxy ethiocholanolone (OHE)

This method was employed to investigate 24 hours samples of urine from 140 normal children and from 34 children with cystic fibrosis aged from 2 months to 15 years

The CF children had normal values for the total 17 keto steroids and for A E, DHA KA and OHA

On the other hand the values for 11 keto-ethiocholanolone (KE) and for 11 hydroxy ethiocholanolone (OHE) were significantly lower in children with CF than in normal children

We have at present no definite explanation for this finding It is striking that reduction of the values for only two out of the four cortisone cortisol metabolites (KA OHA KE OHE) were encounterd in the children with CF This is perhaps expression for a specific enzyme blockage but this is *not* in the androgenic steroid series

Discussion

Svend G Johnsen The method employed is specific and exact I cannot explain the find

ings described in the children with cystic fibrosis Perhaps they are secondary to liver conditions

Erik Thamdrup Have the conditions in patients with liver disease been investigated?

Svend G Johnsen Yes In such cases severe alterations in the relationships between cortisol and androgenic metabolites are found

E. Winge Flensborg Janne Steinrud & E. Lykkegaard Nielsen *Treatment of cystic fibrosis with anabolic agents and in particular the favourable effect on pulmonary function as measured by peak expiratory flow rate*

Twenty nine patients with cystic fibrosis (CF) were treated with series of testosterone dehydroepiandrosterone and/or dianabol

In all of the treated patients aged 14–15 years with one exception a definite increase in gain in weight and growth in height was demonstrated Simultaneously the general condition improved

It is of particular interest that a definite increase in the peak expiratory flow rate occurred during the periods of treatment and stagnation or fall during the control periods The increase in the peak expiratory flow rate was far in excess of the normal increase anticipated in relation to the increase in growth in height during the periods of treatment The effect on the peak expiratory flow rate was observed in both sexes and at all ages between four and 15 years

Future investigations will possibly reveal whether a non specific effect due to increase of the tone of the respiratory muscles is concerned or a more specific effect due to alteration of the chemical physical nature of the bronchial secretions

From a therapeutic point of view this is a most desirable effect in CF patients in whom the main problem is the chronic obstructive pulmonary lesion

I Thygstrup E Hjørring Hansen & E Winge Flensborg Lip biopsies in cystic fibrosis

In 30 patients with cystic fibrosis (CF) 3 mm punch biopsies were taken from the inner surface of the lower lip near the mid line under local anaesthetic block The same procedure was also undertaken in 13 patients suspected to be suffering from CF

The control material consists of lip biopsies from three children with other diseases and 15 autopsy biopsies from children showed histological changes in the glands of the lips

In the 30 children with definite CF typical changes were found in the glands of the lips in 70° (dilatation of the ducts + inspissated material + acinus atrophy)

Out of the 13 children in whom CF was suspected at the time of the biopsy seven were subsequently proved not to have the condition and all of these seven biopsy findings were normal

Out of the six who were still suspected to be suffering from CF at the time of analysis of the results changes in the glands of the lip were found in four

Lip biopsy is not indicated if the diagnosis has been confirmed by other methods of investigation (sweat test and investigation of duodenal secretion) On the other hand lip biopsy may be of assistance in uncertain cases and particularly in cases in which the sweat sodium shows values which the sweat test is normal but where there is clinical evidence for CF

Meeting May 12 1971

J Øster *Abdominal pain headache and growing pains* (To be published independently)

Flemming Rosleff & Flemming Guttler *Excretion of amino acids and phenylalanine and tyrosine metabolites during loading with phenylalanine*

P Olavsgaard A-M Worm Else Andersen & E W Flensborg *Development of the centres of ossification in children with cystic fibrosis*

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The patient material was very heterogeneous because treatment had been undertaken in various places No form of androgen therapy had however been administered

When the skeletal age and the chronological age are plotted graphically 73% of the girls and 53% of the boys fall below the normal scatter The observation that more of the girls do so is in agreement with the fact that cystic fibrosis is a more serious disease in them and with a greater excess mortality On subdividing the patients into degrees of severity from 1–4 (as estimated particularly by the pulmonary changes) it is shown that in boys there is good agreement between severity of the disease and lowest bone age whereas no definite connection could be demonstrated in girls

By comparing the chronological age of the CF patients with 1) the height and 2) weight in relation to a normal material it was found as anticipated that the height and particularly the weight were definitely reduced and, as with the skeletal age, this was most pronounced in girls When the height is considered in relation to the bone age the height is found to be relatively more reduced

Thus, children with cystic fibrosis have apparently retarded skeletal development, height and, in particular, weight These values may therefore be employed to assess the response to therapy

Discussion

Henning Andersen The growth in height is more influenced than the skeletal development which does not support the theory of a specific androgenic defect

Erik Thamdrup The pathological conditions described here are similar to those in other chronic diseases

E Winge Flensborg & Svend G Johnsen *Significant changes in two 17 ketosteroid fractions in cystic fibrosis*

A new method of investigating the 17 ketosteroids in the urine (Girad purifying and g s chromatographic analysis) permits isolation and determination with great accuracy of seven isolated pure 17 ketosteroids androsterone (A) ethiocholanolone (E) dehydroepandrosterone (DHA) 11 keto androsterone (KA), 11 keto ethiocholanolone (KE) 11 hydroxy androsterone (OHA) and 11 hydroxy ethiocholanolone (OHE)

This method was employed to investigate 24 hours samples of urine from 140 normal children and from 34 children with cystic fibrosis aged from 2 months to 15 years

The CF children had normal values for the total 17 keto steroids and for A, E DHA KA and OHA

On the other hand the values for 11 keto-ethiocholanolone (KE) and for 11 hydroxy ethiocholanolone (OHE) were significantly lower in children with CF than in normal children

We have at present no definite explanation for this finding It is striking that reduction of the values for only two out of the four cortisone cortisol metabolites (KA OHA KE OHE) were encounterd in the children with CF This is perhaps expression for a specific enzyme blockage but this is *not* in the androgenic steroid series

Discussion

Svend G Johnsen The method employed is specific and exact I cannot explain the find

Thereafter the results of a prospective investigation of 24 IDM is reported In these infants electrocardiographic monitoring was undertaken during the first three days of life and daily determinations of serum electrolytes acid base status serum protein and blood sugar were undertaken

Out of these infants 80% developed hypocalcaemia and 90% hyperkalaemia One further infant developed a left sided bundle branch block pattern which was normalized following administration of calcium intravenously With this exception only few insignificant electrocardiographic changes were observed No relation could be demonstrated between the degree of the hypocalcaemia and the length of the QT interval Hyperkalaemia was not accompanied by characteristic changes in the T waves

It is concluded that observation of IDM in the first days of life should include frequent determinations of serum electrolytes and electrocardiographic monitoring in the more severe cases so that adequate therapy with calcium and possibly insulin glucose intravenously can be instituted if electrocardiographic changes of the type described here should develop

Discussion

N J Brandt The serum Ca values reported here with one exception are not definitely abnormal for newly born infants

Hyperkalaemia alone may explain the disturbances in rhythm The effect of intravenous calcium is also compatible with changes in rhythm due to hyperkalaemia

Bent Friis Hansen The effect of calcium was dramatic at any rate In addition to calcium and potassium sodium magnesium phosphorus and the pH are of significance for regulation of the cardiac rhythm

B Zachau-Christiansen & M Yssing *Mortality and complications of exchange transfusions ...*

With the object of illustrating the operations mortality and the incidence of complications of exchange transfusions in neonates a review is undertaken of the course of 794 exchange transfusions undertaken in 534 patients in the paediatric department Rigshospitalet during the period 1 V 1961–1 V 1965 The material was subdivided into three diagnostic groups rhesus sensitization (80%) other forms of sensitization or possible ABO sensitization (12%) and hyperbilirubinaemia without sensitization (8%)

A total of 61 complications were registered cerebral symptoms and/or respiratory distress syndrome after the intervention in 35 cases attacks of cyanosis in five cases respiratory arrest in two cases seizures in two cases and technical accidents in 17 cases or 2% of the exchange transfusions The 17 technical accidents consisted of the following unsuccessful catheterization of the umbilical vein (8) blockage of the catheter (5) difficulty in catheterization of the umbilical vein with possible perforation (2) hypervolaemia and dyspnoea towards the conclusion of the intervention (1) and perforation of the umbilical vein followed by fatal intraperitoneal haemorrhage (1)

Nineteen children died during or after exchange transfusion In 3/4 of the infants severe haemolytic anaemia was the main cause of death In three out of the 19 infants brain damage which had occurred prior to the intervention was the cause of death Two of these three had kernicterus In one child the cause of death was a technical accident This was the only death which was due directly to the exchange transfusion in the material which gives an operative mortality of 1 3 per thousand This risk is so slight that it may be ignored when assessing the indications for prophylactic exchange transfusion

The mortality for the total material is 3 6% per child and 2 4% per intervention In the rhesus group the mortality is 4 per child and 2 5 per intervention In the group with other forms of sensitization or possible ABO

It has long been recognized that patients with phenylketonuria (PKU) excrete more phenylalanine in the urine than normal individuals. On the other hand, investigations concerning the excretion of the remaining amino acids have long been neglected.

We have, therefore, investigated the quantitative excretion of amino acids in the morning urine and in the subsequent six hours following oral loading with phenylalanine. The investigation was undertaken by means of ion exchange chromatography and was performed on 11 normal individuals, seven patients with PKU, 13 heterozygotes and four patients with persistent hyperphenylketonuria (HPA).

The investigation revealed that in the unprovoked state, there is hyperaminoaciduria in PKU and HPA patients which is most marked in the case of PKU patients while the heterozygotes have normal or slightly increased total excretion.

Oral loading with phenylalanine results in different excretion patterns in the four experimental groups. In normal individuals excess excretion of the majority of the amino acids significant for valine, isoleucine, tyrosine, phenylalanine and glutamic acid.

In the heterozygotes corresponding excess excretion was encountered of nearly all the essential and non essential amino acids significant for tyrosine, phenylalanine, histidine and serine and alanine. Simultaneously, it was observed that the excess excretion of tyrosine was less than the corresponding excess excretion in normal individuals during the first six hours after loading. This difference was, however, not significant.

In the PKU patients loading resulted in a decrease in essential amino acids (apart from phenylalanine) significant for methionine, leucine, tyrosine, lysine, histidine, serine, alanine and arginine.

The excretion of essential amino acids in HPA patients after loading resembles the state of affairs in normal individuals and heterozygotes. Decrease in threonine and isoleucine were observed but were not significant. The pattern for the non essential amino acids corresponds, on the other hand, to that in PKU as all of the amino acids (with the exception of cystine) were excreted in lesser quantities significant for serine and glutamic acid. It was of particular interest that HPA patients both prior to and after loading excreted considerably less phenylalanine than the PKU children. This is evidence against the theory propounded by Lines & Waismann (1) that HPA patients are virtually phenylketonuric patients with serum contents of phenylalanine of less than 20 mg% on account of an excessive loss of phenylalanine in the urine.

Final interpretation of the excretory conditions must wait until the results of the corresponding plasma investigations are known.

1. Lines, D. R. & Waismann, H. A. *J Pediat 78*: 474, 1971.

Discussion

N. J. Brandt: It is difficult to assess the results when the serum values are not known. In general quantitative estimation of amino acids both in the urine and the plasma is difficult. Intra individual variations are nearly as great as inter individual variations.

J. C. Melchior: Have the HPA patients clinical symptoms?

E. Wamberg: No, they feel perfectly well.

H. T. Lund, Alf Wennevold, B. Friis Hansen & M. Yssing: *Hypocalcaemia and hyperkalaemia as the causes of reversible bundle branch block in newly born infants of diabetic mothers*

Two cases of left sided bundle branch block in newly born infants of diabetic mothers (IDM). Both of these infants had hypocalcaemia and hyperkalaemia. The electrocardiographic changes became normalized in immediate relationship to intravenous administration of calcium.

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Discussion

J Vesterdal These excellent results demonstrate that it pays to centralize What was the mortality among premature infants? What is meant by ABO immunization?

M Yssing We have no figures from recent years during which we have extended the in dications for exchange transfusion so that we now employ this procedure also in small pre mature infants who tolerate it remarkably well The diagnosis ABO immunization was established where this was a possibility and it is thus often a theoretical diagnosis

E Freiesleben From a serological aspect we are unable to contribute towards the diagnosis of ABO immunization We had good experi ence with the Munk Andersen test in the be ginning but gradually we found a number of positive results in cases in which there was no possibility of ABO immunization

P Plum We have reviewed the clinical signi ficance of ABO immunization and found that an infant with blood type A whose mother had blood type 0 has considerably greater risk of developing athetosis

N J Brandt

Acta Pædiat Scand 61 387–388 1972

PROCEEDINGS OF PAEDIATRIC SOCIETIES

FINNISH PAEDIATRIC SOCIETY

Meeting October 23 1971

Pekka Halonen *Virological aspects on measles vaccination*

Marjatta Kunnas (Tampere Finland) *The clinical importance of measles vaccination*

Complications of measles appear especially during pregnancy and in connection with other diseases such as pulmonary diseases and the ones requiring immunosuppressive treatment The most important immediate complications of measles are encephalitis (estimated 1/1 000) and pneumonia while myocardial and urinary tract lesions occur less often The most dangerous late complication known is subacute sclerosing panencephalitis (SSPE)

The measles frequency in Finland has stayed at the same level through the 50 s and 60 s in contrast to the sharp decrease in morbidity in countries where measles vaccination has been undertaken Immunization is nowadays achieved solely by living attenuated virus Measles vaccination ought to be introduced in Finland

Antti Vaheri *Rubella vaccination*

Immunity following natural rubella (German measles) *infection gives* the best prophylaxis against rubella during pregnancy Passive immunization with *gamma* globulin gives some protection but it may mask clinical symptoms modify the immune response and make diagnosis difficult

Attenuated rubella virus strains with rather well established properties as vaccines have now been produced and licensed in North America or Western Europe HPV77/DE5 HPV77/DK12 and Cendehill and RA27/3 (Wistar)

By the summer 1971 about 30 million doses had been used in the USA and several million elsewhere Vaccination of susceptible subjects induces formation of rubella antibodies in over 95% of vaccinees However the titre of serum antibody is considerably lower than that induced by natural rubella There are also qualitative differences between the antibodies induced by natural infection and by vaccines except for perhaps the RA27/3 strain The vaccines give little protection against reinfection but protect against clinical rubella Small amounts of vaccine virus are excreted in the upper respiratory tract but the vaccinee is not contagious Arthritis is a common complication particularly in adult vaccinees (10–50%)

To evaluate the hazard to the fetus of accidental administration of live rubella vaccine during pregnancy the vaccine was given to 24 seronegative women who were certified for legal abortion (Vaheri Vesikari Oker Blom Seppala Parkman Veronelli and Robbins 1969 1971) Rubella virus was recovered from the placenta in six cases and in one case from the fetus Virus was also found in over 50 of uterine cervix swabs taken 9 to 28 days after vaccination The results indicating that vaccine virus may invade the placenta and fetus stress the need to observe the recommended precautions when vaccinating post pubertal female patients

Several hundred instances of inadvertent

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Several hundred instances of inadvertent

vaccination of pregnant women have already occurred In 12 pregnant vaccinees (Center for Disease Control, Report), known to be susceptible, 4 pregnancies were terminated by therapeutic abortion 2 by spontaneous abortion and 6 were carried to term with no gross abnormalities in the newborn In three aborted cases the placenta and/or decidua showed some histopathological changes and virus was isolated

If the present live vaccine strains are introduced two vaccination policies may be followed In the USA mass vaccination of children was initiated in 1969 The aim was to eliminate the main reservoir of rubella virus and eradicate the disease In the UK primarily adolescent girls, 11–14 years of age are vaccinated In addition adult nonpregnant women could be vaccinated, but only on an individual basis and only after a serologic test shows lack of immunity and pregnancy can be excluded for at least 2–3 months after vaccination Recent evidence suggests that immunity after natural infection and perhaps more so after vaccination is maintained by reinfection This is a key question when deciding on the policy for rubella prophylaxis in Scandinavian countries

Olli Koskimies

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC SOCIETY

Meeting Sept 26 1971

S Axtrup *Reflections about the perinatal mortality in Kristianstad*

Perinatal mortality during the years 1956–1963 (I) and 1964–1971 (until July) (II) was compared During period II the newborns and their mothers were treated with more modern methods than during I which has led to an increase in the number of infants who survive The material of Kristianstad is to a certain extent also biased as pregnant women with expected complications are admitted from two hospitals in the neighbourhood

The total number of newborns during I was 10 915 Of these 1 6 % were stillborn and 1 2 died within 7 days i e a perinatal mortality of 2 8 During II 14 436 infants were born 1 1% were stillborn and 0 8 % died within 7 days i e a perinatal mortality of 1 9 %

The modern care of prematures in particular during II was one of the reasons for the decreased mortality Among 445 prematures during I 21 % died and among 702 prematures during II 13 5 died

J Silwer *A follow up study after 5 to 7 years of children with an abnormal neonatal period*

R Berg *How common is pharyngeal incoordination?*

In 1951 Macaulay described the syndrome pharyngeal incoordination (P I) superficially similar to oesophageal atresia but unassociated with gross anatomic defects The syndrome is due to transient failure of neuromuscular incoordination of swallowing laryngeal movements and breathing Since then many cases have been published and the condition can no longer be regarded as very rare Within less than 5 years we have diagnosed eight cases of this syndrome which makes about one per 2 000 deliveries In the severe cases (five of our eight) the symptoms were readily recognised Hoarseness from the first cry was typical The clinical picture was dominated by excessive continuous flow of saliva as a sign of the child s inability to swallow The situation was similar during feeding Aspiration pneumonia was observed in three of our cases These children had attacks of coughing and cyanosis P I is a syndrome without organic obstruction or other serious anatomical defects Congenital defects amenable to surgery must be ruled out The unstable and unduly sensitive nervous system of the newborn and the complex innervation of pharynx with risk of birth injuries (n vagus) were discussed Once the diagnosis has been made treatment offers no difficulties Feeding via a stomach tube results in rapid improvement and may be life saving

R Sundgren *Radiological aspects of catheterization of the umbilical artery and veins*

Catheterization of the umbilical artery and veins for the measurement of blood gases and the administration of infusions during the first weeks of life are required in many cases

to assure accurate diagnosis and adequate therapy

The literature reviewed indicates complications in 4–5% of the cases in the form of thrombosis or sepsis originating in the umbilical veins liver necroses bleeding in connection with catheterization

Since faulty positioning of the catheter can be assumed to account for a number of these complications the praxis of checking the position of the tip of the catheter by injection of contrast medium via catheter was established more than a year ago at the paediatric clinic of the Central Hospital in Kristianstad in cooperation with the department of radiology

These injections of contrast medium have been free from complications and have in a number of cases given valuable information concerning the vessel topography and made possible a more correct positioning of the inserted catheter

B Bergwall & B A Ljungblom *Suprapubic aspiration of the urinary bladder for accurate diagnosis of urinary tract infections in children*

A study was performed to compare the results of urine cultures obtained by suprapubic aspiration (s p a) and clean voided midstream (c v m) in children with suspected urinary tract infections

In 34 children, 2–8 years old with sterile s p a the concurrent c v m coincided provided according to Kass two c v m were taken Five falsely positive cultures were obtained if only one c v m was relied upon In 11 children 2–8 years old the c v m and the s p a both revealed > 100 000 bact /ml urine

In 63 children, 1 week – 2 years old the results of s p a were compared with those when urine was collected in colostomibags 48 of these children has sterile s p a and the bag cultures coincided in 44 cases provided three bag cultures were taken If two bag cultures were relied upon 39 coincide and if only one was relied upon 31 would coincide

The s p a of 15 children revealed positive cultures in a range of < 1 000– > 100 000 bact / ml The concurrent 50 bag cultures coincided with the exception of three negative interpreted as the result of bactericide effect of poorly rinsed disinfectant

It seems justified to rely upon c v m but not on specimens collected in colostomibags The risk of missing infections with < 100 000 bact /ml urine also has to be considered

K B Lykken & J Grogaard *Suprapubic aspiration of the urinary bladder A comparative bacteriologic study*

Suprapubic aspiration of the bladder (vesicopuncture) was performed in 300 patients One case had gross haematuria of 8 hours duration In 122 patients clean voided and vesicopuncture urines were cultured on the same day for comparative bacteriologic study

Group I (Neonatal patients)

The indications for examination were Pathological sediment earlier positive culture failure to thrive icterus vomiting Thirty of 102 patients in the age of 0–1 month had > 10 bact /ml in clean voided urine 8 had positive bladder bacteriuria (73% false positive cultures) Twenty five patients had 10^4–10 bact /ml in clean voided urine 3 (all boys) showed bladder bacteriuria Cultures $> 10^4$ bact /ml in clean voided urine gave 80% false positive cultures Forty seven patients had 10^4 bact /ml in clean voided urine none of them showed bladder bacteriuria

Group II Children above 6 months age

The indication for vesico puncture was recurrent asymptomatic bacteriuria 15 girls (age 1–13 years) 11 had $> 10^5$ bact /ml in clean voided urine and 6 of them had bladder bacteriuria (45% false positive cultures) 5 boys (age 6–30 months) all had > 10 bact / ml in clean voided urine only one of them showed bladder bacteriuria (80% false positive cultures)

With liberal use of suprapubic aspiration of

the bladder a decreased frequency of urinary tract infections was observed from 7 6% (1969) to 4 5° during the first 6 months of 1971 in hospitalized patients

Suprapubic aspiration ought to be used for diagnosis of urinary tract infections during childhood especially during the first year of life The method is simple and safe

S Aronson B Gustafson & N W Svenningsen *Suprapubic aspiration of the urinary bladder*

Concomitant with every suprapubic aspiration (SPA) a clean voided sample of urine was obtained In infants this sample was collected in a polyethylene urinary bag after previous thorough cleansing (bag specimen) In children midstream urine was obtained after proper cleansing (midstream specimen) Both samples were kept and sent chilled from the moment of sampling until they were cultured Uncentrifuged urine was immediately examined for pyuria expressed as white cell count per mm³ using a Burker counting chamber

Indication Bacteriuria of doubtful significance by other methods

Results Firstly the results confirm the indication for SPA as mentioned above thereby avoiding improper overtreatment Bacterial counts above 10- bact /ml were considered pathological

Secondly the white cell count in uncentrifuged urine in SPA samples showed more than 5 cells/mm³ only in infants with urinary tract infection i e bacteriuria in SPA In addition 13 of 16 infants with urinary tract infection had cell counts well above 300/mm³ in their bag specimens whereas less than 300 cells/mm³ were obtained in all cases with contamination of the bag specimens Thus the cell count per mm³ in uncentrifuged freshly voided urine as well as in SPA urine should be re evaluated as a feasible method in urinary examination although a white cell excretion within normal limits never excludes bacteriuria

U Otto *What happens after a suicide attempt?*
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SWEDISH PAEDIATRIC SOCIETY

Meeting October 23 1971

L Kohler & C C Arnoldi *Every day problems in paediatric orthopedics*

K Palmen *Dysplasia of the hip joint during the first years of life not diagnosed in the neonatal period*

B Lindquist *Post graduate training in paediatrics in Sweden*

B Cavell, N Svenningsen T Thulin & B Schersten *Measurement of blood glucose in the neonate using test strips with colour meter*

The performance of an enzyme strip/colourmeter method (Dextrostix Reflectance Meter Ames Co) in measuring blood glucose in the neonate was evaluated in 68 newborn infants As a comparison blood glucose was determined simultaneously in each infant by the laboratory (glucose oxidase method) When used as recommended by the manufacturer the reflectance meter underestimated the blood glucose values by 10–20 mg/100 ml blood Consequently, several cases of falsely low blood glucose were encountered

After calibrating the instrument using the back of a special standardisation strip the correspondence between the two methods was improved, the reflectance meter now overestimating the blood glucose values by 5–10 mg/100 ml blood

Replicated determinations on the same blood specimen showed good agreement At a given blood glucose level as determined by the reference method (glucose oxidase) the readings of the reflectance meter varied considerably between individuals suggesting the interference of extraneous factors

N Svenningsen & B Siesjo *Acid base balance in cerebrospinal fluid in newborn infants*

The results of simultaneous measurements of acid base variables in CSF and arterial blood in neonates are reported The mean differences between CSF and arterial blood pH P_{CO} and HCO_3 in neonates compared to adults are presented in Table I The lower arterial blood pH in neonates is inducing a compensatory rise of CSF bicarbonate, which explains the increased CSF bicarbonate in the neonates In a preliminary study of the acid base balance in CSF in preterm infants with IRDS and *respiratory acidosis* we have found a considerable time lag (more than 36 hours) in the compensatory CSF bicarbonate rise Consequently there is a persisting acidosis in CSF of preterm infants with IRDS These findings implicate new aspects on the pathophysiology and therapy of IRDS

Studies comparing the lactate and pyruvate concentrations in CSF and arterial blood have been performed in 17 infants with severe neonatal asphyxia (Apgar score below 4 including heart rate below 100 beats per minute) and 23 control subjects Specific changes with an elevated CSF lactate/pyruvate ratio were found in the asphyxiated infants Simultaneous arterial blood measurements of these variables

Table 1 *Mean differences between CSF and arterial blood in control subjects*

Adults (Posner Swanson Plum) 35	n	Neonates (Present series) 23
−0 103	pH	−0 075
+9 6 mmHg	P_{CO_2}	+9 4 mmHg
−0 5 mEq/l	HCO	+2 9 mEq/l

did not correlate as well as the CSF measurements with clinical signs and development of the neonatal asphyxia Repeated CSF examinations with measurements of the lactate/pyruvate ratios are of diagnostic value in cerebral hypoxia Presently a follow up study of these infants is in progress in order to determine the prognostic value

G Englesson *Needle biopsy as guidance in cystostatic treatment of renal disease*

H Ekelund S C Nettelblad & G Theander *Experiences of congenital diaphragmatic hernia in Malmo*

H Ahlstrom & B Jonsson *The mechanics of breathing in infants with congenital cardiac defects*

Dynamic compliance and work rate against pulmonary resistance was studied in 12 healthy infants and 7 infants with left to right shunts and pulmonary hypertension Oesophageal pressure was determined via a water filled polyethylene catheter The rate of lung volume change was measured with a body plethysmograph in which the infant was placed with the face projecting out through an airtight sealing diaphragm The infants were sleeping during the investigation Measurements were made at rest and during carbon dioxide induced hyperventilation The signals were on line fed to a computer (PDP 8 Digital Equipment) The computer calculated minute ventilation tidal volumes frequency dynamic compliance pulmonary resistance and the work rate of ventilation It was found to be of utmost importance that the signals for pressure and flow were recorded without distorsion Even minor phase lag of one signal compared to the other will cause great errors especially in determinations of dynamic compliance The error will be greatly influenced by the frequency contents of the recorded events Our equipment was carefully controlled with respect to dynamic behaviour Up to 15 Hz was recorded with less than 3° errors in amplitude The phase shift within this range was less than 15° and equal for both signals at any frequency The preliminary results show no significant difference in dynamic compliance or work rate of breathing between infants with left to right shunts and normal infants with similar body surface area The comparison may be influenced by the higher age of the infants with heart disease Two infants were studied before and after operation (pulmonary banding and ligation of a patent ductus arteriosus) A postoperative fall in work rate of breathing was recorded in these two cases

P Henriksson *Transient tachypnea of the newborn infant*

During a 2 year period (7 000 deliveries) 16 infants showed tachypnea (i e respiratory rate more than 60 per minute) on the first day of life persisting 2 to 5 days IRDS pneumonia meconium aspiration pneumothorax and congenital malformations were excluded as an explanation of the symptoms The tachypnea was in most cases as ociated with mild retractions and some had grunting as well Term infants appropriate for gestational age dominated None were asphyxiated at birth Six pregnancies were pathological Male/female ratio 4 1 One infant showed pathological roentgenogram of the lungs with increased perivascular markings and pleural effusions by six hours of age suggesting interstitial oedema The roentgenograms of the other cases were negative but they were all taken after 24 hours

of age All infants were breathing normally by the fourth day

In similar cases reported by Avery et al (*Am J Dis Child 111* 380 1966) Kuhn et al (*Radiology 92* 751, 1969) Swischuk (*Am J Radiol 108* 557, 1970) and Taylor et al (*Ped Clin N Am 18* 975 1971) the pathogenetic speculations are focused around delayed resorption of or accumulation of fluid in the interstitial tissues of the neonatal lung

N R Lundstrom & W Mortensson *Mitral insufficiency due to a balloonating mitral valve*

During a four year period 8 children aged 4 to 15 years with a syndrome characterized by a midsystolic clique and a late systolic apical murmur have been observed Two patients had Marfans syndrome and three more were tall and slender There has been a great variability of the physical findings with an increase of the systolic murmur in sitting and standing position Five patients developed abnormal electrocardiographic findings during physical exercise

Ballooning of the mitral valve during ventricular systole and mitral regurgitation was demonstrated by cineangiocardiography with contrast injection into the left ventricle In all patients abnormal ultra soundcardiographic echoes from the mitral valve were found

The prognosis was discussed with regard to the possibility of myxomatous transformation of the mitral valve the risk of bacterial endocarditis and a possible risk of sudden death

K Dahlin *Brachial plexus paralysis A follow up study*

Infants with brachial paralysis in the neonatal period were reexamined for evaluation of residual damage 10/16 recovered without deficit 9 of them in less than 2 months One infant died because of associated phrenicus paralysis Splints for abduction are not recommended as they may reinforce tendency for luxation For prevention care must be taken during normal deliveries (9/16 of the presented cases) especially when pressing head down against perineum while the shoulder is caught behind the symphysis

Johan Gentz

NEW BOOKS RECEIVED

B M Kagan & E R Stiehm (eds) *Immunologic incompetence* 396 pp illus Year Book Medical Publishers Inc Chicago 1971 Price not given

Planning and programming for nursing services World Health Organization Public Health Papers No 44 Geneva 1971

Family planning in health services World Health Organization Technical Report Series No 476 Geneva 1971

J G Howells (ed) *Modern perspectives in adolescent psychiatry* 614 pp illus Oliver & Boyd Edinburgh 1971 £9

J Rendle Short *The child A textbook for the paediatric team* 200 pp illus John Wright & Sons Ltd Bristol 1971 £2 25

B Modan *The polycythemic disorders* 177 pp illus. Charles C Thomas Publisher Springfield Ill 1971 US $26 00

E B Singleton & M L Wagner *Radiologic atlas of pulmonary abnormalities in children* 251 pp illus W B Saunders Company Ltd Philadelphia London Toronto 1971 £5 75

A B Bergman J B Beckwith & C G Ray (eds) *Sudden infant death syndrome* 295 pp illus University of Washington Press Seattle London 1970 95 s US $10 00

R Richterich *Klinische Chemie Theorie und Praxis* 632 pp illus S Karger AG Basel 1971 Sfr 98 –

J G Brunson & E A Gall (eds) *Concepts of disease A textbook of human pathology* 1134 pp illus Collier-Macmillan Publishers London 1971 £11 95

B Tennant (ed) *Neonatal enteric infections caused by Escherichia coli* Annals of The New York Academy of Sciences vol 176 405 pp The New York Academy of Sciences New York 1971 US $?8 00

U Krech M Jung & F Jung *Cytomegalovirus infections of man* 124 pp illus. S Karger AG Basel 1971 Sfr 32 –

M Manciaux *Abrégé de pediatrie preventive et sociale* 288 pp Flammarion medecine Paris 1971 Fr 32 –

Health education in health aspects of family planning World Health Organization Technical Report Series No 476 Geneva 1971

H U Zollinger *Pathologische Anatomie* Band I *Allgemeine Pathologie* 336 pp illus. Georg Thieme Verlag Stuttgart 1971 DM 12 80

C B Courville *Birth and brain damage An investigation into the problems of antenatal and paranatal anoxia and allied disorders and their relation to the many lesioncomplexes residual thereto* 408 pp illus Margaret Courville 1000 Oxford Way Pasadena California 91103 USA US $?0 00

E P Issel *Bedeutung der zerebralen Geburtsschaden fur die Entwicklung des Kindes in der Gesellschaft* 215 pp VEB Georg Thieme Leipzig 1971 M 43 50

BOOK REVIEWS

Th Ehrenpreis *Hirschsprung s disease* Year Book Medical Publ Inc Chicago 1970 175 pp US $13 50

This book is the first in a new series on surgical conditions in infancy and childhood edited by Mark M Ravitch The series is designed to give a more detailed authoritative treatment of specific subjects of joint interest to pediatricians and pediatric surgeons than can be given in common textbooks

The author has been very successful in fulfilling the editor s requirements On the basis of many years personal experience of Hirschsprung s disease he provides us with a very comprehensive view of the disease including history pathology pathophysiology diagnosis and treatment The author s analysis of the prognostic importance of different external factors is particularly interesting He stresses the importance of *early* diagnosis and *early* instituted treatment and feels that a variety of approaches can be successful as long as they are complemented at an early stage in the disease The author also gives a detailed description of the different operative procedures used for treatment of Hirschsprung s disease and discusses the choice of operation

The book is richly illustrated and the X ray pictures as well as the drawings are of a very good quality The reference list is extensive and almost everything that has been written about this disease is included

The book will be of great value for everyone who has to take care of patients with Hirschsprung s disease

S C Nettelblad

R E Gross *An atlas of childrens surgery* Saunders Co Philadelphia London and Toronto 1970 191 pp illus £8 1s 6d

In this atlas Dr Gross has presented his own methods of performing the most common operations in the general surgery of infancy and childhood and in the repair of congenital cardiovascular abnormalities The different operations are demonstrated in a series of drawings in which the procedure is followed step by step Every drawing is accompanied by a short explanatory text The drawings are very well done being detailed but not overly so and they provide a continuity so that one can follow the different procedures from the positioning of the patient on table and the incision to the closing of the wound The text is short and clear which makes it easy for everyone to review a given procedure in short time A drawback is that the author very seldom suggests an alternative procedure which might be suitable in some situations

This book should be added to the library of every surgical department where operations on infants and children are performed

S C Nettelblad

H J Kaufmann (ed) *Progress in pediatric radiology Vol 3 Genito urinary tract* S Karger A G Basel 1970 380 pp illus DM 96 —

Like its two predecessors in this series the present volume serves to elucidate a specific area within the vast field of pediatric radiology by offering the contributions of several distinguished authors from various countries The topics include the main diagnostic procedures such as urography micturition urethrocystography renal angiography vena-cavagraphy and genitography as well as their application in certain disease entities e g congenital anomalies tumours pyelonephritis and renovascular hypertension Other chapters are concerned with the radiation exposure due to urinary tract diseas and with the radiographic anatomy of the human fetus as illustrated in intra uterine studies with contrast media

The reader is stimulated not only by the lucid account of knowledge and experience but also by the differences in approach between some of the contributors as reflected by modifications of procedures and slight divergencies in opinion and emphasis Invited comments to a special treatment article on the lower urinary tract direct attention to some of these divergencies and thereby further increase the merit of this useful publication

Georg Theander

PRENATAL DIAGNOSIS OF CHROMOSOME ABNORMALITIES

A J THERKELSEN G BRUUN PETERSEN O R STEENSTRUP J JONASSON
J LINDSTEN and L ZECH

From the Institute of Human Genetics University of Aarhus the University Maternity Hospital Aarhus Denmark the Department of Clinical Genetics Karolinska Hospital Stockholm and the Institute for Medical Cell Research and Genetics Medical Nobel Institute Karolinska Institutet Stockholm Sweden

The technique of prenatal analysis of the human chromosome complement has lately been developed to such an extent that prenatal investigation of the karyotype of a foetus can be done early in the second trimester of pregnancy with a high success rate As late as in 1969 the interval between amniocentesis and chromosome analysis was stated to be six weeks by Dancis (4) In 1970 Nadler & Gerbie (9) published the results of the investigation of a total of 160 amniotic fluid samples in 155 of which karyotype analysis was done with an average interval between amniocentesis and successful chromosome analysis of 14 2 days

The following is a presentation of a series of prenatal chromosome investigations The results show that chromosome analysis can be done with a success rate close to 100 and with an interval between amniocentesis and chromosome analysis of less than 14 days

MATERIAL AND METHODS

Amniocentesis was done by the abdominal route in the midline between the umbilicus and symphysis after local anaesthesia of the abdominal wall but without any attempt to localize the placenta before hand In most cases a No 22 gauge lumbar needle was used A series of 45 samples was taken from 36 rhesus immunised women in the third trimester of pregnancy Another series of 18 samples was taken because of various genetical indications from 18 women pregnant in the second trimester Generally 10 ml of amniotic fluid was used for preparing the cell cultures The cultivation of the amniotic cells was done in the following way

(*a*) Centrifugation of the amniotic fluid at 100 × g for 10 min

(*b*) Removal of the supernatant and resuspension of the cells in 2 ml of medium (70% Eagle s medium (Gibco BME diploid) with 20 foetal calf serum (Flow Laboratories) and 10% human AB serum)

(*c*) Addition of 1 ml of the cell suspension to each of two Leighton tubes Bubbling with 5 CO in air before stoppering and incubation at 37 C

(*d*) Change with 2 ml of fresh medium one of the cultures at day 2 and the other at day 3 The old medium is transferred to another Leighton tube and 1 ml of fresh medium is added All four Leighton tubes are subsequently changed every second or third day

Initially the preparations for chromosome analysis were done in different ways but the method of choice was the following

(*a*) Incubation of the cultures (primary cultures if possible) with Colcemide® (Ciba) at a final concentration of 10 g/l for four hours

(*b*) Trypsinisation with 1 ml of 0 05% Trypure® (Novo A S Copenhagen) for 3 min The procedure is repeated if necessary

(*c*) Washing of the cells with Hanks balanced salt solution hypotonic treatment with 0 3% NaCl solution fixation and preparation by air drying technique

Fluorescence analysis of chromosomes (2) was done in all cases with structural rearrangements

This study was supported by grants from the Danish State Research Foundation the Research Foundation of the University of Aarhus Denmark and from Expressen s Prenatal Research Fund

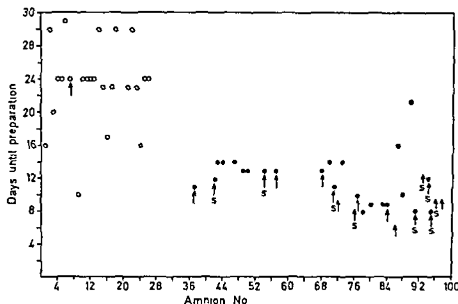


Fig 1 Number of days from amniocentesis until the preparation of chromosomes in the 18 second trimester (↑) and the 41 third trimester cases The samples marked with an *S* were sent from Stockholm to the laboratory in Aarhus ● 1st passage in Leighton tubes × 2nd passage in Leighton tubes ○ 2nd passage in 100 ml prescription bottles

RESULTS

In one of the 18 second trimester cases (case No 97) and in two of the 45 third trimester cases (Nos 43 and 73) grossly bloody taps were obtained In all three cases the karyotype of the foetus was different from that of the mother Furthermore, a slight admixture of red blood cells was observed after centrifugation in 11 of the 18 second trimester cases and in 8 of the third-trimester cases

The cultivation of amniotic fluid cells was successful in 43 of the 45 third trimester samples and in all the 18 second trimester samples The two third trimester samples that did not grow were contaminated one in the hospital and one in the laboratory Chromosome analysis was possible in 41 of the 43 third trimester samples that grew and in all the 18 second trimester samples

The interval between amniocentesis and the preparation of chromosomes appears from Fig 1 In the beginning preparations were made from second passage cultures, but it was later found to be faster and easier to use the primary cultures A total of 14 third trimester samples and 17 second trimester samples were treated in this way, and the average interval between amniocentesis and preparation of the cultures for chromosome analysis was 12 8 days for the third trimester samples, and 10 5 days for the second trimester samples

The indications for amniocentesis in the 18 second trimester cases, the gestational age at amniocentesis as well as the result of the analysis appear from Table 1

Abortus provocatus was done only in cases 7 and 54 In case 7 the result of the karyotype analysis in the amniotic cell cultures was confirmed in cultures of foetal skin from the abortus The indication for abortion was in this case that the mother was a known carrier of the gene for haemophilia A In case 54 an abortion was induced without awaiting the result of the chromosome analysis because the patient had a verified rubella infection in the 8th week of pregnancy Only one chromosome break was found in the 30 cells analysed In two additional cases the patient was a carrier of the gene for haemophilia A but in both cases the foetus was found to be female

In a total of six cases the patient or her husband was a carrier of a balanced translocation either a 14/21 translocation (cases 37 68, and 76) a 15/19 translocation (case 91 see Fig 2) a 1/22 translocation (case 93 see Fig 3) or a 1/13 translocation (case 97 see Fig 4) In two of these six cases the foetus had a normal karyotype (cases 68 and 97) whereas a balanced translocation was found in four (37, 76 91 and 93) In the remaining cases the investigations were made because the parents were nervous about having a child with Down's

Table 1 *Indications and results for the second trimester cases*

Case no	Gestational age (weeks)	Indication for amniocentesis	Karyotype of foetus	Frequency of tetraploid metaphases ( )
7	15	Patient carrier of haemophilia A	46 XY Dp+s+	13
37	14	Husband carrier of D G translocation	45 XX −14 −21 t(14 q 21 q)+	20
42	17	Patient carrier of haemophilia A	46 XX	14
54	16	Patient rubella 9th week of pregnancy	46 XX	8
57	14	One previous child with Down s syndrome Fear of recurrence though her karyotype was normal 30 years old	46 XY	21
68	14	Patient carrier of D G translocation	46 XY	21
71	18	One previous child with Down s syndrome Fear of recurrence though her karyotype was normal 24 years old	46 XX[a]	8
72	15	Patient carrier of haemophilia A	46 XX	7
76	19	Husband carrier of D/G translocation	45 XX −14 −21 t(14 q 21 q)+	5
77	2?	One previous child with Down s syndrome Patient now 43 years old	46 XY	17
84	13	One previous child with Down s syndrome Patient 46 XX Gp+ aged 24 Husband 46 XY Gp+s+	46 XY Gp+s+ Gp+	18
68	15	One previous child with Down s syndrome Two abortions 24 years old	46 XX	7
91	18	Patient carrier of balanced 15/19 translocation	46 XY t(15p+ 19q−)	10
91	19	Patient s husband carrier of balanced 1/2? translocation	46 XY t(1q− 22q+)	10
94	18	Patient fearing child with Down s syndrome 4? years old	46 XY	27
95	20	Patient fearing child with Down s syndrome 41 years old	46 XY[b]	5
96	18	Patient fearing child with Down s syndrome 42 years old	46 XY	6
97	16	Patient carrier of balanced 1/13 translocation One previous child born with an unbalanced karyotype	46 XX	3

[a] Female twins were born
[b] Male twins were born

syndrome either because they had got one previously (cases 57 71 84 and 86) or because the mother was over 40 years of age (cases 94 95 and 96) or for both reasons (case 77)

In most cases only 15 diploid metaphases were counted and analysed In addition the frequency of tetraploid metaphases was estimated in all the second trimester cases by counting a total of 100 cells (Table 1) The tetraploid cells seemed to be of foetal and not maternal origin which could be seen in the cases where the karyotypes of the mother and the foetus were different (see Fig 5) The tetraploid metaphases were not taken into account at the evaluation of the karyotype of the foetus

In all the third trimester cases the child has been born at the present time All children were normal and the sex was in agreement with the karyotype prenatally determined In the 16 second trimester cases going to term the child has been born in all cases except the two last in Table 1 (cases 96 and 97) The karyotypes determined prenatally were confirmed postnatally in all the cases where a balanced translocation was found (cases 37 76 91 and 93) In one of these cases (case 76) the child had a gross brain malformation

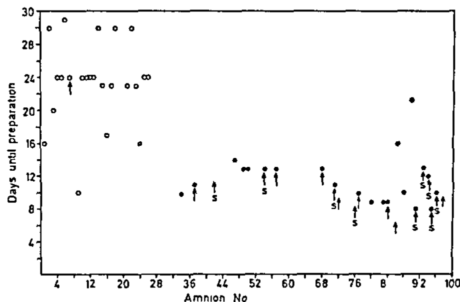


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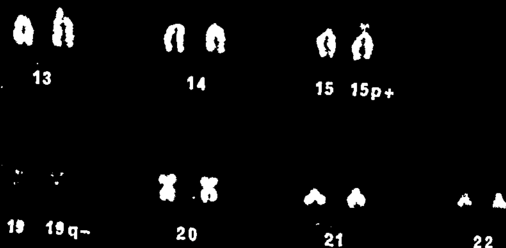


Fig 2 Fluorescence pattern in case 91 (mother) of the chromosomes in groups D F and G A 15 19 translocation of the typ $t(15p+\ 19q-)$ is seen

localized to the region of the third ventricle but no other malformations

All the other children born were normal and their sex was in agreement with the karyotype determined prenatally In cases 71 and 95 the patients gave birth to dizygotic female and monozygotic male twins respectively

DISCUSSION

The results presented show that cultivation of amniotic fluid cells obtained by amniocentesis in the second trimester of pregnancy can be done with a success rate close to 100% and with an interval between amniocentesis and karyotype analysis below 14 days

When deciding on the various indications for a prenatal chromosome analysis the main factor to take into consideration is the risk for the mother and foetus involved in performing the amniocentesis As mentioned above an isolated brain malformation was found in the child born in our case 76, but it is unlikely that this malfo mation should be causally related to the amniocentesis It probably developed at an earlier time

The risk is mostly known from amniocenteses done in the third trimester and the literature on the subject has been reviewed by Burnett & Anderson (1) In 8 280 amniocenteses there were no cases of maternal death Nine cases of foetal death or abortion occurred but the causal relationship between these events and amniocentesis was uncertain Serious complications caused by injuring the foetus with the needle were not seen however Isoimmunisation of the foetus as a result of foeto maternal transfusion caused by the amniocentesis has been claimed to occur but there is some controversy in the literature in this respect (10)

The risk involved in doing a transabdominal amniocentesis therefore seems very low indeed in the third trimester and might very well be lower in the second trimester as a relative hydramniosis exists in this trimester (12)

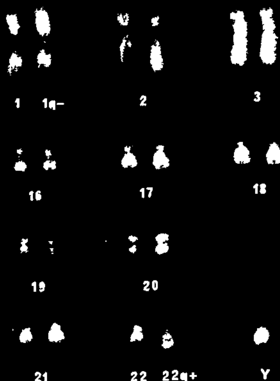


Fig. 3. Fluorescence pattern in case 93 (foetus) of the chromosomes in groups A, E, F and G. A 1/21 translocation of the type t(1q−; 21q+) is seen.

Gerbie et al. (5) have performed transabdominal amniocentesis in a total of 408 women in the second trimester of pregnancy without any maternal or foetal complications.

On basis of these results it is reasonable to assume that the risk is very small and at least below 1% (9). If the risk of foetal death or abortion is of the same size as in the third trimester it should be about one per thousand if all the foetal deaths reported by Burnett & Anderson (1) were due to the amniocentesis which seems unlikely.

It therefore seems justified to perform a prenatal karyotype analysis in pregnant women who have previously given birth to a child with Down's syndrome when the parents fear of recurrence is great, even if their karyotypes are normal. The risk in such cases has been estimated to be between 1 and 2% from a material of mothers below 30 years of age (11).

Prenatal chromosome analysis also seems justified in pregnant women over 40 years of age who have an increased risk of getting children with an extra chromosome. In such cases the risk of a child with Down's syndrome may also be estimated at 1–2% (7).

In cases where one of the parents is a carrier of a balanced translocation the risk of an abnormal child is unknown in rare translocation types as illustrated by cases 91, 92 and 97 in our material. In contrast, the risk

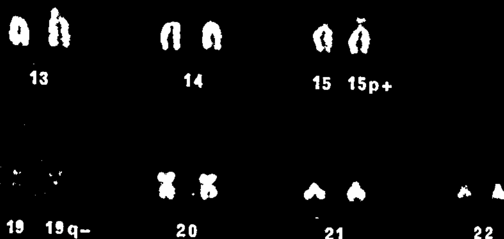


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The risk is mostly known from amniocenteses done in the third trimester and the literature on the subject has been reviewed by Burnett & Anderson (1) In 8 280 amniocenteses there were no cases of maternal death Nine cases of foetal death or abortion occurred but the causal relationship between these events and amniocentesis was uncertain Serious complications caused by injuring the foetus with the needle were not seen however Isoimmunisation of the foetus as a result of foeto maternal transfusion caused by the amniocentesis has been claimed to occur but there is some controversy in the literature in this respect (10)

The risk involved in doing a transabdominal amniocentesis therefore seems very low indeed in the third trimester and might very well be lower in the second trimester as a relative hydramniosis exists in this trimester (12)

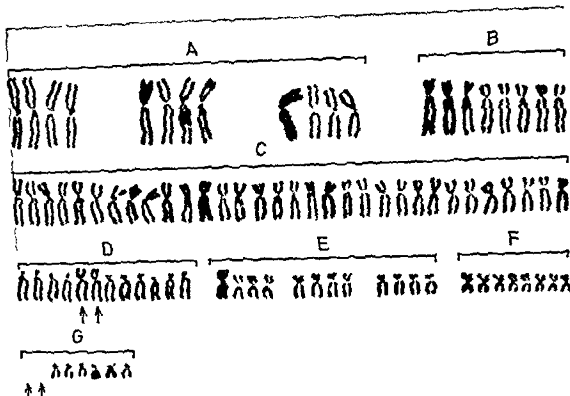


Fig 5 Tetraploid metaphase from Li cul u e of amniotic fluid cells in case 37 The cell ould not be maternal as the *paternal 14/21 translocation* chromo- *om* is *present* The tran location chromosomes and the missing chromosomes in the G group are marked with arrows

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Another cause of failure in the prenatal karyotype analysis may be the admixture of maternal macrophages but according to Nad ler & Gerbie (9) these cells die after one week in culture A third cause is *tetraploidy* which occasionally has been reported to occur with a frequency of 100 in cultures from foetuses with a normal postnatal karyotype (14) There fore *tetraploid cells* have to be disregarded *in* the prenatal chromosome analysis *A foetus* with tetraploid diploid mosaicism will then be diagnosed as normal but since only one in *fant* with a diploid tetraploid mosaicism has been reported *(6) this risk must* be regarded as very small Liveborn children *with* pure tetraploidy have never been reported

SUMMARY

Prenatal karyotype analysis was made of cultured amniotic cells in 41 out of 45 third trimester amniotic samples and in all out of 18 second trimester samples

The third trimester samples were obtained from rhesus immunized women The second trimester samples were taken for various ge netical reasons and include six cases in which one of the parents was a translocation carrier i e one 1/13 one 1/22 three 14/21 and one *15/19* translocation

The average interval between amniocentesis *and chromosome* preparation was 12 8 and 10 5 days *for the* third and second trimester samples respectively

The frequency of tetraploid *metaphases was* determined in the second trimester *samples* and varied between 3 and 27 %

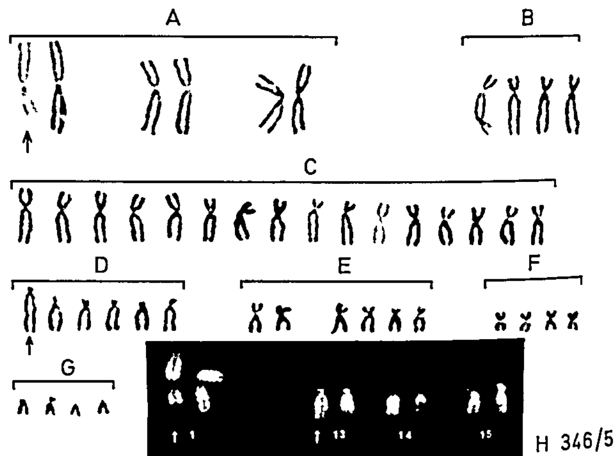


Fig 4 Karyotype of the mother in case 97 showing a balanced A/D translocation which by fluorescence microscopy is seen to be of the type $t(1q-\ 13q+)$ The abnormal chromosomes marked with arrows.

of having a child with Down s syndrome in the relatively common D/G translocations is about 10% when the mother is the carrier and probably less than 5% when the father is a carrier (8)

In cases where the mother is a carrier of the gene for haemophilia A prenatal karyo type analysis is not strictly necessary as the sex of the foetus is revealed by investigations of X and Y chromatin in the amniotic cells with out cultivation However we think that a karyotype analysis should be done if possible as it makes the diagnosis safer and also distin guishes between e g normal males and females with a 45,X0 karyotype, and because hetero morphic fluorescence regions might simulate a Y body (3)

In one of our cases there was no genetical indication for the prenatal karyotype analysis but the investigation was made because the patient had had a rubella infection in the 8th week of pregnancy Only one chromatid break was found among 30 cells analysed which is in agreement with previous cytogenetic studies on abortuses from rubella infected mothers (13) However these findings cannot yet be evaluated since no isolation of rubella virus was made in any of the cases

As mentioned above the sex has been con firmed after birth in most of our second trimester cases and in all our third trimester cases Thus the reliability of the prenatal karyotype determination is very high as is also shown by other authors (5 9) Special difficulties are connected with twin pregnan cies in which amniotic fluid may only be ob tained from one of the twins Our series in cludes one monozygotic and one dizygotic twin pair but as the dizygotic twins were both normal and like sexed the prenatal diagnosis

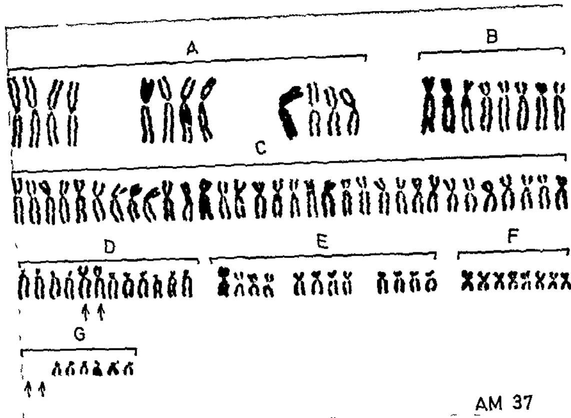


Fig. 5. Tetraploid metaphase from the culture of amniotic fluid cells in case 37. The cell could not be maternal as the *paternal* 14/21 translocation chromosome is *present*. The translocation chromosomes and the missing chromosomes in the G group are marked with arrows.

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The average interval between amniocentesis and chromosome preparation was 12.8 and 10.5 days for the third and second trimester samples respectively.

The frequency of tetraploid metaphases was determined in the second trimester samples and varied between 3 and 27 %

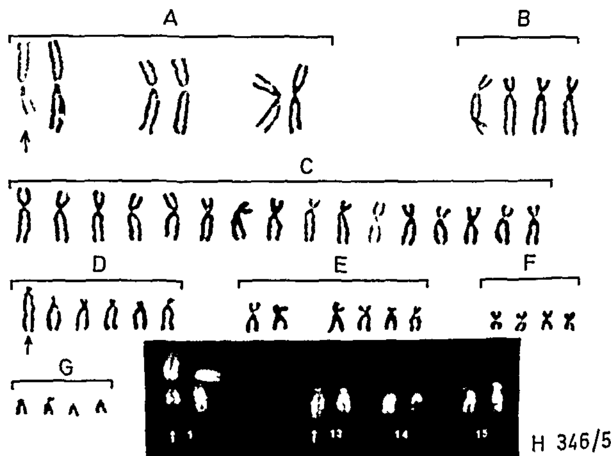


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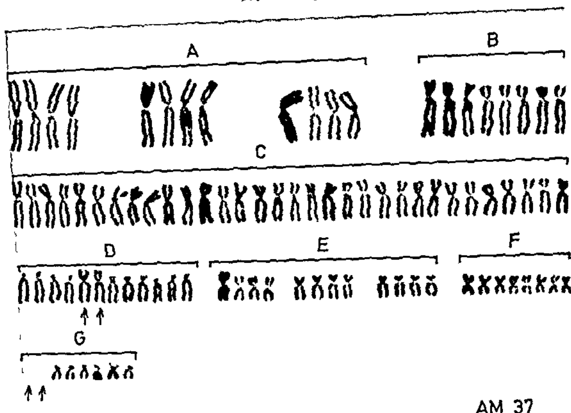


Fig 5 Tetraploid metaphase *from the culture* of amniotic fluid cells in case 37 The cell could not be maternal as the paternal 14/21 translocation chromosome is present The translocation chromosomes and the missing chromosomes in the G group are marked with arrows

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ACKNOWLEDGEMENT

We are greatly indebted to Drs N Hahnemann Margareta Mikkelsen E Niebuhr and J Philip who sent us cases 86 68 84 and 37 respectively for diagnosis

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UNCONJUGATED AND CONJUGATED BILIRUBIN IN PLASMA FROM PATIENTS WITH ERYTHROBLASTOSIS AND NEONATAL HYPERBILIRUBINEMIA

ARNT WINSNES and DAG BRATLID

From the Paediatric Research Institute Rikshospitalet Oslo Norway

The physiological hyperbilirubinemia in neonates as well as the hyperbilirubinemia of prematurity have by most authors been found to be due exclusively to an increase in the unconjugated bilirubin fraction (34 41) In the case of erythroblastosis however both unconjugated and conjugated bilirubin can be found in serum although the majority of these patients (70–90%) show an increase of unconjugated bilirubin only (11 21 22 25 35–37)

Several authors have however claimed that there is a temporary rise in conjugated bilirubin in serum of neonates before the total serum bilirubin begins to fall (6 9 24 42) Some of these results (6 42) have later been challenged (23) as being due to methodological errors

Recently Bakken (3–5) presented data indicating that conjugated bilirubin though normally absent at birth accumulated in serum of neonates and reached a maximum of 2 mg/ *100 ml at* day 4–6 in full term children and 3 mg 100 ml at day 6 in the prematures This last value was also reached by infants with erythroblastosis but at different age depending on the degree of immunization as judged by the indirect Coombs titres in the mothers The most heavily immunized babies had increased amounts of conjugated bilirubin even at birth These studies suggested that an accumulation of unconjugated bilirubin in serum "triggered bilirubin UDP glucuronyltransferase (UDP glucuronate glucuronyltransferase E C 2 4 1 17) whereas an accumulation of conjugated bilirubin in serum triggered the excretion mechanism for conjugated bilirubin

Though these hypotheses were attractive doubt has been raised against some of the underlying data since the direct spectrophotometry method (16) employed for estimation of conjugated and unconjugated bilirubin has been found to be subject to errors (8) If conjugated bilirubin is normally present in serum of neonates in the amounts reported (3–5) this could have clinical implications A reinvestigation of this problem therefore seemed desirable Since conjugated bilirubin was found earlier in the more immunized patients than in normal newborns and the highest levels of conjugated bilirubin coincided with the maximum total bilirubin concentration analysis of plasma obtained at exchange transfusions should offer the best chance of confirming the presence of conjugated bilirubin if present in significant amounts Several methods (based on diazo-coupling chloroform extraction and direct spectrophotometry respectively) have been used for estimation of conjugated and unconjugated bilirubin

MATERIALS AND METHODS

The plasma samples were obtained at exchange transfusion from the first blood withdrawn The syringe

Table 1 *Bilirubin fractions determined in plasma samples from patients with AB0 incompatibility (cases 1-15) and hyperbilirubinemia (cases 16-19)*

Total bilirubin (15) direct reacting bilirubin (34) chloroform extractable bilirubin at pH 2 and 6 respectively (8) conjugated bilirubin according to Van Roy & Heirwegh (38) as well as the percentage of unconjugated bilirubin according to Fog & Bakken (16) were determined

Case	Birth weight (g)	Age (days)	Plasma bilirubin mg/100 ml		Chloroform-extractable bilirubin mg/100 ml		Conjugated bilirubin mg/100 ml v R & H	Unconjugated bilirubin spectro photometry
			Total bilirubin	Direct reacting bilirubin	pH 2	pH 6		
1	3 130	1	18 4	2 9	17 4	16 9	0 0	107
		2	19 2	1 5	—	18 2	0 0	107
2	2 700	5	28 9	1 8	27 5	27 8	0 2	104
3	2 910	4	23 7	2 2	22 4	23 1	0 1	96
4	3 880	2	22 2	1 4	21 5	22 8	0 1	90
		3	20 8	2 0	20 5	20 4	0 0	97
5	4 240	3	18 7	2 8	17 8	17 8	0 3	91
6	3 010	5	22 6	2 3	21 6	22 8	0 1	83
7	3 580	3	19 2	2 3	20 4	19 5	0 0	87
8	2 600	4	19 3	2 0	21 2	21 2	0 2	90
9	2 810	2	14 2	1 4	16 5	16 1	0 1	82
10	3 200	3	18 3	1 4	19 3	18 9	0 1	96
11	2 050	6	18 5	1 2	20 2	—	0 1	92
12	4 750	3	21 4	2 1	22 5	22 5	0 1	103
13	3 000	1	22 7	2 9	19 8	21 4	0 0	99
14	3 250	1	11 0	0 8	10 2	9 8	0 1	101
15	2 700	4	25 4	0 6	25 3	23 7	0 1	93
16	2 010	1	14 1	3 1	14 6	12 4	0 2	94
17	1 600	5	22 8	2 2	20 5	21 6	0 0	101
18	2 100	4	18 4	2 0	18 2	18 3	0 0	103
19	4 150	3	21 7	1 3	21 6	23 0	0 1	105
Mean	3 035	3	20 1	1 9	20 0	19 9	0 1	96

used for blood withdrawal contained heparin giving a final concentration of 125-150 I U /ml in the sample The blood sample was stored in the dark at about 5 for not more than 20 hours before the blood corpuscles were sedimented by centrifugation and the plasma pipetted off The plasma samples were stored in the dark at - 15 until analysis

31 plasma samples from 23 Rh immunized patients with highest indirect Coombs titres in the mothers in the range 128-8 196 17 plasma samples from 15 pa tients with ABO incompatibility 3 samples from as many premature babies as well as 1 sample from a full term newborn with hyperbilirubinemia were ana lysed

The degree of intra uterine affection was measured according to Liley (29)

Total bilirubin was determined according to Jen drassik & Gróf as modified by Fog (15) Conjugated bilirubin was measured by the method of Van Roy & Heirwegh (38) and direct reacting bilirubin by the method of Nosslin (34) Chloroform extractable bili rubin at pH 2 and pH 6 was determined as described by Bratlid & Winsnes (8) and unconjugated bilirubin was determined by direct spectrophotometry as de scribed by Fog & Bakken (16)

The indications for exchange transfusion were as described by Aagenæs (1)

RESULTS

Most of the exchange transfusions because of Rh erythroblastosis (Tables 2 3) were per formed during the first day of life whereas exchange transfusions because of ABO in compatibility and hyperbilirubinemia without immunization (Table 1) were performed in pa tients up to 6 days old (mean 3 days)

The mean total bilirubin in patients with hyperbilirubinemia and ABO incompatibility (Table 1) was 20 1 mg/100 ml and the mean direct reacting bilirubin was 1 9 mg/100 ml This value can be explained solely by the inevitable reaction of a small part of the un conjugated bilirubin and therefore gives no indication of the presence of conjugated bili rubin when plotted in the correction scheme (Fig 1) according to Nosslin (34) As described elsewhere (8) the difference between the total bilirubin and the chloroform extractable bili rubin at pH 2 probably gives an estimate of

the amount of bilirubin diglucuronide present whereas the difference between the chloroform extractable bilirubin at pH 2 and pH 6 probably gives an indication whether bilirubin monoglucuronide is present or not None of these methods gave any indication of the presence of bilirubin conjugates (Table 1) The recently developed specific method for determination of conjugated bilirubin by diazo-coupling with ethylanthranilate (38) gave very low values mean 0 1 mg/100 ml (Table 1) The direct spectrophotometry method indicated the presence of 4° conjugated bilirubin (Table 1) which is far below the values obtained by analysis of heel prick samples (3–5)

Patients with Rh-erythroblastosis and indirect Coombs titres in the mothers of 512 or below (Table 2) showed a mean total bilirubin of 8 6 mg/100 ml with a mean direct reacting value of 1 2 mg/100 ml According to Nosslin (34) this value gives no indication of the presence of conjugated bilirubin (Fig 1) The chloroform extraction procedures at pH 2 and pH 6 indicated the possible presence of small amounts of conjugated bilirubin whereas the diazo coupling with ethylanthranilate gave low values mean 0 1 mg/100 ml The direct spectrophotometry method indicated the presence of 10 conjugated bilirubin

Patients with Rh erythroblastosis and indirect Coombs titres in the mothers from 1 024–8 196 (Table 3) showed a mean total bilirubin of 9 3 mg 100 ml with a mean direct reacting bilirubin value of 1 8 mg/100 ml not indicative of the presence of conjugated bilirubin (Ref 34 Fig 1) Significant amounts of conjugated bilirubin were not found with the chloroform extraction procedures The diazo-coupling with ethylanthranilate indicated the presence of 0 2 mg/100 ml The direct spectrophotometry method indicated the presence of 14 conjugated bilirubin (Table 3) This is above the value found in the less immunized group (Table 2) and definitely above the percentage found in patients with ABO incompatibility and hyperbilirubinemia (Table 1) This difference may however be explained

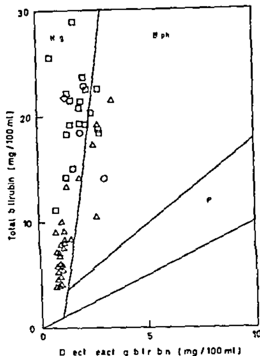


Fig 1 The values for direct reacting plasma bilirubin are plotted according to Nosslin (34) The plasma dilution prior to analysis usually was 10-fold though at lower levels of total bilirubin a 5 fold dilution was used in some cases The symbols represent the following conditions in the patients △ Rh-erythroblastosis □ ABO-erythroblastosis ○ hyperbilirubinemia of prematurity and ◇ hyperbilirubinemia of unknown etiology in a full term child

by the sensitivity of the direct spectrophotometry method for hemoglobin admixture (8) since increased amounts of heme pigments are found in the sera of Rh immunized patients (2 7)

Judged by the mean values obtained with the colorimetric methods used in the present study (Tables 1–3) significant amounts of conjugated bilirubin could not be detected in the analysed samples However some of the methods definitely indicated the presence of bilirubin conjugates in some of the samples Fig 1 shows that 9 of the samples (representing 8 patients) showed biphasic reaction according to Nosslin (34) Two of these samples came from a heavily Rh immunized patient (Table 3 case no 20) who died 3 days old with kernicterus diagnosed at autopsy This patient also

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Case	Birth weight (g)	Age (days)	Plasma bilirubin mg/100 ml Total bilirubin	Plasma bilirubin mg/100 ml Direct reacting bilirubin	Chloroform-extractable bilirubin mg/100 ml pH 2	Chloroform-extractable bilirubin mg/100 ml pH 6	Conjugated bilirubin mg/100 ml v R & H	Unconjugated bilirubin spectro-photometry
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16	2 010	1	14 1	3 1	14 6	12 4	0 2	94
17	1 600	5	22 8	2 2	20 5	21 6	0 0	101
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Table 3 *Bilirubin fractions determined in plasma samples from patients with moderate to severe Rh erythroblastosis*

Plasma samples were obtained from patients whose mothers had indirect Coombs titres in late pregnancy at 1024 and higher levels The methods for bilirubin determination were the same as cited in Table 1

Case	Birth weight (g)	Max titres indirect Coombs	Liley zone	Age (days)	Plasma bilirubin mg/100 ml		Chloroform extractable bilirubin mg/100 ml		Conjugated bilirubin mg/100 ml v R & H	Unconjugated bilirubin spectro-photometry
					Total bilirubin	Direct reacting bilirubin	pH 2	pH 6		
10	2 850	1 074	II	1	7 0	0 9	6 3	7 1	0 1	96
11	? 700	1 0 4	II	1	9 1	1 2	9 9	9 8	0 0	8(
l	2 700	1 074	I	1	3 8	0 7	3 7	3 8	0 0	84
13	3 070	1 074	II	1	7 9	1 0	7 7	6 7	0 1	88
14	1 920	1 0 4	II	1	12 9	2 1	13 5	13 9	0 1	76
15	? 450	1 074	—	1	5 5	0 8	5 2	5 1	0 2	75
				1	8 0	1 0	7 6	7 5	0 1	75
16	3 460	2 048	II	1	14 1	1 9	13 9	14 1	1 0	104
17	3 3 0	? 048	II	1	5 2	0 8	4 8	4 9	0	97
18	1 730	2 048	I	1	10 0	0 9	10 0	9 7	0 2	75
				1	15 0	1 4	14 6	13)	0 1	107
19	2 930	2 048	I	1	4 0	0 9	3 9	3 9	0 1	97
0	? 80	4 096	—	1	10 5	2 7	10 3	10 1	0 1	100
				3	21 5	3 5	23 3	-?1	0 8	74
1	3 050	4 096	II	1	4 0	0 9	4 1	—	0 0	69
2	? 300	4 096	II	1	4 9	0 9	5 1	5 1	0 0	77
				1	9 0	0 8	8 7	9 2	0 0	102
				2	19 2	2 8	18 9	18 3	0 1	9?
23	2 870	8 19?	II	1	5 9	1 0	5 9	5 9	0 0	65
Mean	_ 749	—	—	1	9 3	1 8	9 3	9 5	0 2	86

globin admixture is minimal though not absent) in the present study whereas Bakken used heel prick blood specimens where hemoglobin admixture is inevitable (31)

The occurrence of bilirubin in the urine as revealed by the Ictotest has been taken as indicative of conjugated bilirubin in the serum of neonates (3 5) In our opinion this is not necessarily so The Ictotest is very sensitive (and subjective) being positive at bilirubin concentrations of 0 05–0 10 mg/100 ml Since a slight amount of albumin can be found normally in the urine (12) amounts of unconjugated bilirubin enough for qualitative demonstration by Ictotest might be excreted in the urine when the total bilirubin concentration exceeds a certain limit In fact considerable amounts of unconjugated bilirubin have been found in urine of newborn infants (26) and employing the quantitative method of Michaelsson (30) values in the range 0 1–0 5 mg/100 ml are often found in hyperbilirubinemic newborn infants (30 and own unpublished observations)

Although great amounts of conjugated bilirubin could not be detected in any of the analysed samples some of the methods indicated the presence of bilirubin conjugates in a few cases (Tables 1–3 Fig 1) This is in agreement with several authors who have found increased direct reacting bilirubin levels in serum of some patients with erythroblastosis (11 21 22 25 35–37)

Most authors give a fixed upper normal limit for the direct reacting bilirubin concentration above which conjugated bilirubin is thought to be present The amount of unconjugated bilirubin that will react directly increases however with increasing total bilirubin concentrations (34 37) It is therefore necessary to use a correction scheme as suggested by Nosslin (34) whose method we recommend for the clinical use It should how

Table 2 *Bilirubin fractions determined in plasma samples from patients with mild to moderate Rh erythroblastosis*

Plasma samples were obtained from patients whose mothers had indirect Coombs titres in late pregnancy not higher than 512 The methods for bilirubin determination were the same as cited in Table 1

Case	Birth weight (g)	Max titres indirect Coombs	Liley zone	Age (days)	Plasma bilirubin mg/100 ml		Chloroform extractable bilirubin mg/100 ml		Conjugated bilirubin mg/100 ml v R & H	Unconjugated bilirubin spectro photometry
					Total bilirubin	Direct reacting bilirubin	pH 2	pH 6		
1	3 230	128	III	1	7 0	0 9	6 4	6 2	0 1	89
2	3 620	256	II	2	17 3	2 6	18 0	18 1	0 1	102
3	2 965	256	II	1	7 5	1 1	7 4	7 3	0 1	92
4	3 460	256	II	1	10 0	1 1	9 7	8 8	0 2	86
5	3 700	256	—	1	7 1	0 9	7 3	6 8	0 2	99
				4	13 3	1 4	10 3	10 0	0 1	94
6	4 010	256	II	1	8 3	1 2	8 6	8 4	0 2	88
7	2 500	256	III	1	8 3	1 5	7 7	7 7	0 1	102
8	2 900	256	II	1	5 5	1 0	5 5	5 1	0 0	82
				1	6 0	0 9	5 8	5 9	0 0	96
				1	8 0	1 2	7 1	7 4	0 1	96
9	1 910	512	—	1	4 6	0 8	4 5	4 6	0 1	67
Mean	3 143	—	—	1	8 6	1 2	8 2	8 0	0 1	90

had increased level of conjugated bilirubin as judged by the diazo coupling with ethylanthranilate (Table 3) The results obtained by the chloroform extraction procedures although giving nice mean values varied greatly making the interpretation of the single results difficult Increased amount of conjugated bilirubin was also found in another Rh immunized patient (Table 3 case no 16) with ethylanthranilate diazo coupling procedure

DISCUSSION

The present study shows that with the use of colorimetric methods significant amounts of conjugated bilirubin are usually not found in the plasma of patients with Rh erythroblastosis (0–4 day old) and ABO erythroblastosis (1–6 days old) This conclusion is in contrast to that of Bakken (3–5) but in agreement with most other reports on neonatal hyperbilirubinemia (34 41) The presence of such small amounts of conjugated bilirubin (0 09 mg/100 ml at maximum) as reported by Brodersen et al (9 24) employing an isotope derivative method is however, in good agreement with the values obtained in the present study with the method of Van Roy & Heirwegh (38)

The results obtained with the direct spectrophotometry method indicating the presence of 10–14% (mean values) of conjugated bilirubin in plasma from patients with Rh erythroblastosis (0–4 day old) are similar to those of Bakken (3–5) However these values probably are artefactual As reported elsewhere the direct spectrophotometry method gives falsely high values of conjugated bilirubin when heme pigments are present in the sample (8) Since heme pigments are regularly found in plasma of patients with erythroblastosis (2 7) this can therefore explain the high levels of conjugated bilirubin found with the direct spectrophotometry method

In plasma from patients with ABO incompatibility and hyperbilirubinemia only 4% (mean) conjugated bilirubin was found on day 3 with the direct spectrophotometry method (Table 1) compared with approximately 20% on the same day in full term and premature babies as reported by Bakken (3–5) These discrepancies probably are explained by the use of exchange transfusion plasma (where hemo

discussed together with the clinical significance of determination of conjugated bilirubin in neonatal hyperbilirubinemia

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ever be concluded that determination of small amounts of conjugated bilirubin is difficult with the colorimetric methods used in this study The new and specific diazo coupling method of Van Roy & Heirwegh (38) may prove useful although more experience with this method seems to be required before it can be recommended for clinical purpose

The significance of bilirubin production and the different steps in bilirubin elimination for the development of neonatal hyperbilirubinemia have been the subject of extensive investigation (41) Most authors agree that the neonatal hyperbilirubinemia cannot be due to increased hemolysis and increased bilirubin production (13 40) Studies on laboratory animals indicate that both hepatic uptake of bilirubin (19, 28), bilirubin conjugation (10 14 17 18 20 27) and excretion of the bilirubin conjugates (17) proceed at a reduced rate in the neonatal period Direct evidence concerning the relative importance of these different steps in bilirubin elimination in man is however scarce Bilirubin UDP glucuronyl transferase activity in three prematures (post mortem liver) was zero or very low (27) The studies of Vest (39) indicated that also excretion of i v injected glucuronides proceeded at a reduced rate in neonates However the present report as well as those of others indicate that either hepatic uptake or conjugation of bilirubin normally is the rate limiting step since conjugated bilirubin is found only in a small group of neonates Since conjugated bilirubin though is present in some patients it is possible that the excretion capacity for conjugated bilirubin is just great enough to handle the amount of conjugated bilirubin produced and that little or no reserve capacity exists Severe erythroblastosis might adversely affect the excretory mechanism thus making this step the rate limiting one This interpretation is supported by the fact that the classical inspissated bile syndrome (22) is found only in the more severely affected patients with erythroblastosis (11 21 22 25 35, 36)

It was assumed that the bilirubin UDP glucuronyltransferase normally is inactive at birth (3–5) since conjugated bilirubin in serum did not occur before the 2nd or 3rd day of life Occurrence of conjugated bilirubin in serum certainly indicates that the bilirubin UDP glucuronyltransferase is active, but failure to demonstrate bilirubin conjugates does not necessarily imply that enzyme activity is absent Conjugated bilirubin in significant amounts would not be expected to be present in serum unless the conjugation capacity exceeded the capacity of the excretory mechanism Higher amounts of conjugated bilirubin in the serum of patients with erythroblastosis than in serum of full term babies or prematures do therefore not necessarily indicate that the former have a higher bilirubin UDP glucuronyltransferase activity as suggested (3–5)

From the present paper it must be concluded that conjugated bilirubin is normally not found in clinically significant amounts in the sera of newborns and the directions for therapy can therefore usually be based on the total bilirubin concentration However, some patients definitely have some conjugated bilirubin in their sera and in patients with inspissated bile syndrome determination of this bilirubin fraction is of clinical significance Because of the low sensitivity and degree of accuracy of the colorimetric methods available the evaluation of slightly elevated values for conjugated bilirubin is however difficult

SUMMARY

52 plasma samples obtained at exchange transfusion from patients with Rh and ABO immunization and hyperbilirubinemia of prematurity were analysed for the presence of conjugated bilirubin Five different methods were used It was concluded that significant amounts of conjugated bilirubin are usually not present in the plasma of neonates with hyperbilirubinemia In view of these findings the relative importance of the different steps in bilirubin elimination in the newborn is

MAXIMAL TUBULAR REABSORPTION OF GLUCOSE IN INFANTS AND CHILDREN

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Glucose is freely filtered at the glomerulus and almost completely reabsorbed by the proximal tubular cells (14 31 49) In normal subjects only trace amounts of glucose can be detected in the final urine (1 2 11 19 26 28 32 33) The transport mechanism is active specific for glucose and carrier mediated (8 36 37 48) Its capacity is generally considered to be rate limited as originally described by Shannon & Fisher (35) in dogs and the maximal rate was termed tubular maximum for glucose (Tm_G) by Smith et al (38) In recent years however much evidence has accumulated that a fixed tubular maximum does not indeed exist for glucose but that reabsorption depends on many factors including GFR (15 20 46) intratubular flow rate (6 22 23) fluid reabsorption (6 41) and extracellular fluid volume (29)

There are relatively few data in the literature on normal values of Tm_G in infants and children (15 17 39 43 44) In our laboratory glucose loading was performed in infants and children for various diagnostical reasons in order to determine the Tm_G Of these 8 infants and 16 children were found after thorough investigation to be free of any tubular and metabolic disturbances and to have normal glomerular filtration rates Their values can be considered normal and the data from these children will be presented here As will be shown Tm_G corrected to surface area of adults increases with age while the ratio Tm_G/C_I is the same in infants and children Furthermore Tm_G is correlated positively with the inulin clearance rates in all subjects It is also correlated with plasma glucose concentrations in infants but not in older children

METHODS

Tm was determined according to the standard procedures described by Stalder (39) following the determination of inulin clearance (C) Blood glucose was elevated by intravenous administration of 40% glucose in a dose of 0 8 g/kg bodyweight followed by intravenous infusion of 20 glucose at a rate of 1 2 g/min/1 73 m Urine was collected through an indwelling bladder catheter and Tm values were determined after 60 min of equilibrium in 2 consecutive urinary collecting periods of 15–20 min Capillary blood (finger tip) for glucose determination was withdrawn before and after urinary collecting period in order to avoid painful stimuli during the clearance periods All other procedures of clearance techniques were the same as described before (5)

The 8 infants were 2 / to 24 weeks old and the 16 children 1 / to 13 years In preliminary communications the values of 9 infants and 33 children were reported Some of those cases were excluded here since a simultaneous loading with phosphate and/or PAH had been performed in those subjects a procedure which might have interfered with glucose reabsorption In the present report therefore glucose loading was performed exclusively

Glucose was determined in capillary blood and urine by glucose oxidase (Biochemica Test® Boehringer) The urines were diluted several times and no

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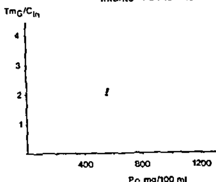


Fig 2 The correlation betwe n Tm_G/C_I and plasma glucose concentration at which Tm was determined in infants (see text)

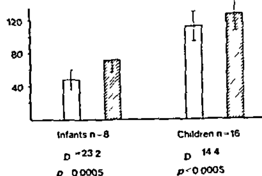


Fig 4 The effect of hypertonic glucose upon glomerular filtration rate in infants and children

these variables ($Y = 0\ 0039X + 0\ 203$ $r = 0\ 85$ $p < 0\ 001$) while in children the correlation is not significant ($r = 0\ 35$ $p < 0\ 1$) This finding may be the clue for great variations in Tm_G values reported for infants in the literature It is noteworthy that such relationship seems to be non existent in children

The infusion of hypertonic glucose produced significant changes in glomerular filtration rate As shown in Fig 4 the increase in C_I is much more pronounced in infants than in children in both absolute and relative terms C_{IAH} also increased significantly from a mean 554±75 to a mean of 619±115 ml/min/1 73 m² ($p < 0\ 0001$) in 11 children it was not determined in infants

The serum electrolytes were lowered as the consequence of hypertonic glucose administration as is shown in Table 2 while the osmolality of the serum increased significantly from a mean of 272 to 292 mosm/kg H_2O The increase of osmolality never exceeded the level of 30 mosmol/kg H O which might be considered to be potentially dangerous

The clearance rates of sodium chloride potassium phosphate and the osmolar clearance measured in children only increased significantly during hypertonic glucose administration (Table 3) while the free water clearance (C_{H_2O}) dropped from 5 07 to −2 13 ml/min/1 73 m The mean urinary volume increased slightly and non significantly in infants from 6 9 to 8 7 and in children from 8 7 to 10 8 ml/min/1 73 m²

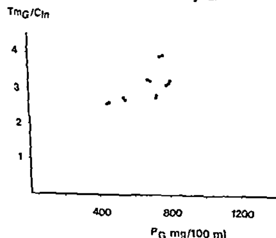


Fig 3 The values of Tm /C plotted against plasma glucose concentrations in children (see text)

DISCUSSION

In Table 4 the normal values of Tm_G and Tm_G/C_I reported in the literature are compared with those obtained in the present investigation There are great variances in the mean values and their standard deviations or

Table 1 *Maximal tubular glucose reabsorption in 8 infants (2½–24 weeks) and in 16 children (1½–13 years)*

Mean values ($\bar{X}$) standard deviations (S D) and ranges are given for inulin clearance (C_{In}) plasma glucose concentration (P_G) maximal tubular glucose reabsorption (Tm_G) and the ratio Tm_G/C_{In}

	C_{In} (ml/min/ 1 73 m²)	P_G (mg/100 ml)	Tm_G (mg/min/ 1 73 m²)	Tm_G/C_{In} (mg/ml)
Infants				
$\bar{X}$	72	701	213	2 94
S D	15	62	71	0 74
Range	61–95	560–980	147–318	2 02–4 30
Children				
$\bar{X}$	126	650	362	2 83
S D	19	131	96	0 47
Range	95–160	427–835	227–557	2 28–3 47

inhibition of GOD by substances present in undiluted urines could occur Inulin in serum and urine was determined by a microadaption of the recorcinol method (30) after glucose in the samples was oxidized to gluconic acid by GOD The method u ed was developed in our own laboratory before the hexokinase method became available Later on it was found that an almost identical method had been described by Froesch et al (13) The following procedure was done in our laboratory Deproteinization of 50 μl serum with 100 μl ZnSO (10) and 100 μl NaOH (0 5 N) incubation for 60 min at 37 C of 50 μl of supernatant with 50 μl GOD solution (0 5 mg GOD I No 15423 Boehringer in 1 ml phosphate buffer pH 5 6 Soerensen) then determination of inulin with recorcinol A standard curve with inulin and a serum blank was run in each det rmination

Tm₃ was calculated according to the standard formula $Tm = P_G \times C_I - U_G V$ (mg/min/1 73 m²) In each ubject two clearance periods were used for calculation The surface areas were calculated ac cording to the formula of DuBois and DuBois (Geigy Tabellen)

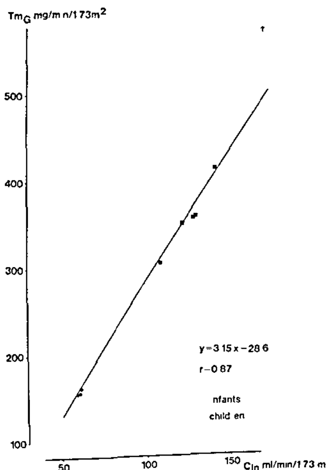


Fig 1 The correlation between maximal tubular glucose reabsorption (Tm_G) and glomerular filtration rates (C_I) in infants and children

RESULTS

The results obtained in infants and children are listed in Table 1 which shows the mean values, standard deviations and ranges of inulin clearance (C_{In}) plasma glucose concentrations (P_G) maximal tubular glucose reabsorption (Tm_G) and the ratio of Tm_G/C_{In} Mean Tm_G is significantly lower in infants than in children ($p<0\ 001$) while Tm_G/C_{In} is the same in both age groups The levels of plasma glucose obtained during glucose infusion were high enough to obtain load/Tm_G ratios of more than 1 5 thus assuring a complete saturation in each case

In Fig 1 the individual value of Tm_G are plotted against C_I Each individual determination from all the infants and children are given and a positive correlation between Tm_G and C_I was observed The least-squares regression line can be described by the equation $Y=3\ 15X-28\ 6$ and the correlation is highly significant $r=0\ 87$ $p<0\ 0005$ demonstrating the dependence of Tm_G on glomerular filtration rate This does not necessarily mean a causal relationship but could be produced by simultaneous changes in kidney functions in the process of maturation

In Figs 2 and 3 the ratio Tm_G/C_I is plotted against plasma glucose concentration (P_G) in infants and in children respectively In infants there is a positive correlation between

ranges This might partly be explained by different methods of glucose determinations as indicated in the table Furthermore simultaneous loading with PAH or diodrast and glucose titration may also influence tubular glucose transport mainly via extracellular fluid volume expansion

In *children* Tm_G values seem to be the same as in adults although direct comparisons with the same methods are not reported Infants usually are considered to have lower rates of Tm and Tm_G/C_{In} indicating tubular immaturity and glomerular preponderance in this respect There are however only two reports (15 43) available to support this view In the report by Tudvad no exact figures are given neither for Tm_G nor GFR and furthermore "no actual attempts were made at determining the glucose Tm in that study It therefore does not seem justified to draw definite conclusions from those early findings Gekle and coworkers also claim to have found tubular immaturity in the infants which they examined Their values for Tm_G/C_I seem indeed to be low They have however presented only two infants who showed exceptionally low values of Tm_G/C_I while the other 6 showed the same normal values as 2 children who also were investigated by these authors A gradual increase of Tm_G/C_I in relation to age is therefore not evident from their data Furthermore the linear correlation between Tm_G and GFR postulated by both Tudvad and Gekle is in contradiction with the assumption of maturational change in the tubular parameter It is therefore concluded that the figures given in the literature do not suffice to postulate an immaturity of tubular glucose reabsorptive mechanism in relation to glomerular filtration in fullterm infants beyond the age of 2–3 weeks This is in full agreement with our findings The net tubular glucose reabsorption (Tm) on the other hand certainly is lower in infants which has to be related to low GFR in this period of life

It is not our intention to reject the concept of tubular immaturity for glucose reabsorption completely This may indeed be present in the newborn period and in prematures but its existence has not been proved so far In prematures a great variance in Tm_G values was reported by Tudvad & Vesterdal (44) but their mean Tm_G/C_{In} was not lower than those ratios in children or adults reported by other investigators (see Table 4) Since Tudvad & Vesterdal gave no normal values for children or adults examined with their own method, it remains questionable whether they really have demonstrated a significant reduction of Tm_G/C_I in their 7 premature infants as usually cited in the literature

In the present study a positive correlation was found between Tm_G and C_I in different infants and children This is in agreement with early findings of Smith et al (38) in adults later reaffirmed by Vitelli et al (47) and by Gekle et al (15) in infants It also was shown by physiologists in dogs (7 18 20 27) and in rats (46) Recalculations of even those reports which claimed to show no correlation between Tm_G and GFR demonstrate such a correlation as van Liev et al (46) have shown These findings indicate that Tm_G is not an independent fixed tubular function but that it depends on other kidney functions including GFR The values of Tm_G may be modified by different variables At cellular level however a true Tm_G can be demonstrated as very recently Tune & Burg (45) have shown on isolated rabbit nephrons

A positive correlation between Tm_G/C_I and plasma glucose concentration was demonstrable only in infants but not in children It may be speculated that the low glomerular filtration rates of infancy could be responsible for this phenomenon as experimental data (18 46) suggest Van Liev et al (46) found higher rates of Tm_G/C_I in rats with GFR below 0 3 ml/min/g kidney than in those with GFR above that limit and Handley et al (18) found higher Tm_G/C_I values in dogs when GFR was acutely reduced by dehydration

Furthermore the state of extra cellular fluid volume may have influenced the reabsorption

Table 2 *Effect of hypertonic glucose on blood electrolytes in children*

	Before glucose	During glucose	$\bar{X}_D$	p	N
P_{Na} (mEq/l)	137 8 ± 4 0	131 5 ± 5 4	6 3	< 0 0005	11
P_{Cl} (mEq/l)	100 4 ± 2 5	96 1 ± 3 7	4 3	< 0 0005	11
P_K (mEq/l)	4 25 ± 0 42	3 70 ± 0 38	0 55	< 0 0005	11
P_P (mg/100 ml)	4 69 ± 0 50	3 60 ± 0 55	1 09	< 0 0005	16
P_{osm} (mosm/kg H_2O)	272 1 ± 6 7	292 4 ± 4 7	−20 3	< 0 0005	7

Table 3 *Effect of hypertonic glucose on electrolyte and osmolar clearances in children* (*all va* *in ml/min/1 73 m²*)

	Before glucose	During glucose	$\bar{X}_D$	p	N
C_{Na}	1 16 ± 0 61	3 41 ± 2 50	− 2 25	< 0 0005	11
C_{Cl}	1 12 ± 0 50	3 80 ± 1 43	− 2 67	< 0 0005	11
C_K	20 3 ± 8 9	19 9 ± 8 2	+ 0 4	< 0 49	11
C_P	12 0 ± 7 1	19 6 ± 7 5	− 7 6	< 0 0005	16
C_{osm}	2 99 ± 0 58	14 66 ± 2 13	− 11 67	< 0 0005	7
C_{H_2O}	+ 5 07 ± 4 04	− 2 13 ± 3 46	+ 7 21	< 0 0005	7

Table 4 *Normal values of Tm_G and Tm_G/GFR reported in the literature*
H J = Hagedorn Jensen   GOD = glucose oxidase   Hex = hexokinase

	Tm_G (mg/min/1 73 m²)	Tm_G/GFR (mg/ml)	N	Comments methods
Adults				
Smith et al 1943	375 ± 80[a]	2 70	24 male	Simultaneous diodrast loading in most cases
	303 ± 55[a]	2 53	11 fem	
Govaert et al 1949	160-380 mg/min	2 41 ± 0 35[a]	45	
Brod 1964	361 ± 82[a]	3 31	8	Anthrone
McPhaul et al 1968	325 ± 36[a]	2 34 ± 0 21[a]	16	GOD
Elsas et al 1969	291 ± 27[a]	2 38 ± 0 40[a]	7	GOD
Children				
Stalder 1960	304 ± 55[a]	2 22	19 (4–16 y)	H J
	401 ± 28[a]	2 94	7 (4–16 y)	GOD
Grossmann et al 1968	254 ± 115[a]	1 82	65 (3–15 y)	GOD simultaneous PAH loading
Brodehl et al 1971	362 ± 96[a]	2 83 ± 0 47[a]	16 (1–13 y)	GOD
Infants				
Tudvad 1949	120-200[b]	no data given	4 (15–40 d)	H J
Gekle et al 1967	36–288	0 90–2 38	8 (1/2–8 m)	Hex
Brodehl et al 1971	213 ± 71[a]	2 94 ± 0 74[a]	8 (1/2–6 m)	GOD
Prematures				
Tudvad 1949	25–190[b]	no data given	15 (1–95 d)	H J
Tudvad et al 1953	59–175	2 31 (1 90–3 18)	7 (1/2–4½ m)	H J simultaneous PAH loading

[a] 1 standard deviation (±1 S D)
[b] Approximate values since no exact figures are given

ranges This might partly be explained by different methods of glucose determinations as indicated in the table Furthermore simultaneous loading with PAH or diodrast and glucose titration may also influence tubular glucose transport mainly via extracellular fluid volume expansion

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Furthermore the state of extra cellular fluid volume may have influenced the reabsorption

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P_K (mEq/l)	4 25±0 42	3 70±0 38	0 55	<0 0005	11
P_P (mg/100 ml)	4 69±0 50	3 60±0 55	1 09	<0 0005	16
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of glucose, as already shown experimentally for glucose (29), as for phosphates (12, 40, 42) and bicarbonates (21, 25) Measurements of extra cellular volume could not be performed in the present study It may be speculated, that those infants with higher plasma glucose levels who showed higher rates of Tm_G/C_{In} had developed less expanded extra cellular volume than those with lower plasma glucose levels, inspite the fact that both received equal amounts of glucose and fluid per kg bodyweight

Finally what is the meaning of determining Tm_G in clinical medicine? As mentioned above, Tm_G values dependent on many factors and are the result of multiple variables It is, therefore, not surprising to find those high variations in the reported values Single measurements of Tm_G are of no great significance In order to eliminate at least one variable it is necessary to determine the ratio Tm_G/C_{In}, instead of Tm_G which allows comparisons between infants children and adults Measurement of Tm_G/C_{In} should be performed under strict standard conditions and if possible be associated with determination of extra cellular volume, in order to standardize the results It is self-evident that these procedures are too laborious and burdensome to be used widely in clinical medicine

Tubular dysfunction remain the dominant indication to examine tubular glucose transport Here the same precautions have to be observed It is obvious that many controversial results concerning the renal threshold for glucose (T_G/C_{In}) and the maximal reabsorption in these states can be explained by changes in those variables mentioned above Strict standardized procedures are necessary in order to obtain unequivocal results

SUMMARY

Maximal tubular glucose reabsorption (Tm_G) was measured in 8 infants ($2^1/_2$–24 weeks of age) and 16 children ($1^1/_2$–13 years) Mean value of Tm_G in infants was 213 ± 71, and in children 362 ± 96 mg/min/1 73 m The ratios of Tm_G/C_{In} were the same in infants ($2\,94 \pm 0\,74$) as in children ($2\,83 \pm 0\,47$ mg/ml) From data of the literature and from the present study it is concluded that there is no evidence of more enhanced immaturity in tubular glucose reabsorption in infants beyond the age of 2–3 weeks Low net Tm_G values are due to low kidney function with low glomerular filtration rate at this age

A positive correlation was found between Tm_G and C_{In} Additionally, in infants there was a positive correlation between Tm_G/C_{In} and plasma glucose concentrations which was not observed in children These findings demonstrate that Tm_G is not an independent fixed tubular function but may be modified by other factors including GFR and extracellular volume

ACKNOWLEDGEMENTS

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Finally, what is the meaning of determining Tm_G in clinical medicine? As mentioned above Tm_G values dependent on many factors and are the result of multiple variables It is therefore not surprising to find those high variations in the reported values Single measurements of Tm_G are of no great significance In order to eliminate at least one variable it is necessary to determine the ratio Tm_G/C_I instead of Tm_G which allows comparisons between infants children and adults Measurement of Tm_G/C_{In} should be performed under strict standard conditions and if possible be associated with determination of extra cellular volume, in order to standardize the results It is self evident that these procedures are too laborious and burdensome to be used widely in clinical medicine

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SUMMARY

Maximal tubular glucose reabsorption (Tm_G) was measured in 8 infants (2 1/2–24 weeks of age) and 16 children (1 1/2–13 years) Mean value of Tm_G in infants was 213±71 and in children 362±96 mg/min/1 73 m The ratios of Tm_G/C_{In} were the same in infants (2 94±0 74) as in children (2 83±0 47 mg/ml) From data of the literature and from the present study it is concluded that there is no evidence of more enhanced immaturity in tubular glucose reabsorption in infants beyond the age of 2–3 weeks Low net Tm_G values are due to low kidney function with low glomerular filtration rate at this age

A positive correlation was found between Tm_G and C_{In} Additionally in infants there was a positive correlation between Tm_G/C_{In} and plasma glucose concentrations which was not observed in children These findings demonstrate that Tm_G is not an independent fixed tubular function but may be modified by other factors including GFR and extracellular volume

ACKNOWLEDGEMENTS

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Acta Pædiat Scand 61 421–425 1972

ZINC IN THE DIET OF HEALTHY PRESCHOOL AND SCHOOL CHILDREN

C SCHLAGE and BIRGIT WORTBERG

From the Forschungsinstitut fur Kinderernahrung Dortmund BRD

Zinc is regarded as an essential trace element in human nutrition (14) Growth retardation and hypogonadism are most apparent signs of zinc deficiency in man (15) An increasing number of enzyme systems are found to be dependent on zinc (9 24) Monographs and review articles dealing with zinc in human nutrition have been published recently (9 15 23) Uncertainties still exist about the dietary requirement of zinc in human nutrition

In spite of an increasing number of data on the zinc content of foods (18) calculations of zinc intakes from diets are still of limited value In the course of long term studies on nutrient intakes of healthy children fed *ad libitum* on a conventional diet we analysed the zinc contents of daily food intakes of preschool and school children

MATERIAL AND METHODS

In the course of one year 149 daily food intakes of 11 children between 3 and 13 years of age were analysed During the study periods the preschool children received their food in the kindergarten of the Forschungsinstitut fur Kinderernahrung in Dortmund The school children stayed in a nearby municipal children s home The dietary pattern of this home is known to us from former studies ranging over several years (5) and conforms with the diet given to the preschool children Both groups of children received a varied mixed diet of the pattern usual in Westfalia

Supported by the Ministerium fur Wissenschaft und Forschung (Landesamt fur Forschung) des Landes Nordrhein Westfalen

Ages sex and selected anthropometric data of the children under study are given in Table 1

During the meals the children were served by dieticians or specially trained nurses The plates the amounts of food the children asked for and any returns were weighed Aliquots of the amounts of foods actually eaten were collected from the surpluses prepared Aliquots of snacks and drinks taken between the meals were added into the daily food composite These daily food samples were roughly mixed with a wooden spoon and freeze-dried The dried samples were homogenized in an electric mixer for about / minute packed and sealed in airtight plastic bags

Duplicates of 2 5 g each were mineralized in Kjeldahl flasks with HNO /HClO and made up to volume The zinc content was determined by atomic absorption spectrophotometry (AAS) using standard methodology (2) and recalculated for the daily intake There was no contact with zinc-containing material during the preparation of the sample Contamination during the mineralization was compensated for by running reagent blanks along with each batch of samples From 50 duplicates the standard deviation of the method was calculated (10) as 0 040 mg/100 g dry matter the coefficient of variation was 1 8 % Recoveries averaged to 97% (90 %–105) Similar data were found in a methodological study on the determination of zinc in foodstuffs (17) by AAS

RESULTS

Data on the zinc intakes are summarized in Table 2 The preschool children (3–5 years) had about 6 mg zinc per day the school children (10–13 years) had about 10 mg zinc per day There is a considerable day to day variation of the zinc intakes the maximum being up to five times the minimum intake The extreme differences are to quite an extent com

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Table 1 *Data on the children participating in the study*

Child	Sex	Age[a] (years)	Weight[a] (kg)	Height[a] (cm)	Surface[a b] (m^2)	Number of weeks	Number of days
D S	m	2 81	17 5	97 2	0 6695	2	13
J R	f	3 16	14 6	96 4	0 616	6	34
S S	f	4 11	16 2	107 2	0 684	1	7
G K	m	4 52	19 5	113 2	0 783	2	14
S C	f	4 95	18 7	110 3	0 755	1	5
T G	f	5 06	18 7	113 2	0 769	1	7
J S	f	9 67	42 5	139 6	1 269	1	7
S M	f	11 28	32 6	146 3	1 172	3	18
A R	f	11 80	33 2	138 2	1 134	1	4
R R	f	12 75	41 5	156 0	1 362	5	33
O M	m	13 42	33 4	137 7	1 134	1	7
Total 11						24	149

[a] Average for the period studied
[b] Body surface (cm^2) = weight 0 425 × height 0 725 × 71 84 (3)

pensated for by averaging over a period of one week only

The average zinc intakes of the different children and of the two age groups are related to body weights body surfaces and to the energy contents of the daily food intakes The children had between 0 2 and 0 4 mg zinc per kg body weight and day As with other nutrients we find a tendency of decreasing intakes per body weight with increasing age No such tendency can be seen with the intakes if expressed per body surface

The zinc contents of the diets ranged from 1 7 to 9 0 mg/1 000 kcal The averages for the different children varied between 3 9 and 5 2 mg zinc/1 000 kcal

DISCUSSION

With breastfed newborns, intakes of 0 7 to 5 0 mg zinc per day are reported resulting in negative balances (4) (Table 3) Due to the rapidly decreasing zinc content of breast milk the zinc intakes of infants are decreasing ra

Table 2 *Zinc intakes of children per day per calorie intake per weight and per body surface*

Child	Age (years)	Sex	Weeks	Zinc Intakes: mg/day average	Range of intakes mg/day: Per day	Range of intakes mg/day: Average per week	mg/1000 kcal	mg/kg	mg/m^2
D S	2 8	m	2	5 81	3 78– 7 76	5 70–5 92	3 98	0 332	8 67
J R	3 2	f	6	5 32	3 52–11 27	4 55–6 09	4 14	0 364	8 64
S S	4 1	f	1	7 09	4 54– 7 98	—	4 72	0 438	10 36
G K	4 5	m	2	7 42	5 09–11 08	6 08–8 76	4 23	0 380	9 48
S C	5 0	f	1	6 68	4 31– 9 59	—	4 04	0 357	8 84
T G	5 1	f	1	5 81	3 94– 7 40	—	4 07	0 311	7 55
J S	9 7	f	1	9 35	5 04–16 88	—	5 18	0 220	7 37
S M	11 3	f	3	9 69	5 22–16 36	7 73–13 46	5 05	0 297	8 27
A R	11 8	f	1	8 73	2 85–14 96	—	3 91	0 263	7 70
R R	12 8	f	5	10 65	5 59–18 29	9 43–12 06	5 18	0 257	7 82
O M	13 4	m	1	12 37	6 46–18 88	—	4 89	0 370	10 91
3–5 years average				6 36	3 52–11 27	4 55– 8 76	4 20	0 364	8 92
		S D		0 83			0 27	0 044	0 94
9–13 years average				10 16	2 85–18 88	7 73–13 46	4 84	0 281	8 41
		S D		1 42			0 54	0 056	1 43

Table 3 *Data on daily intakes of zinc per day per body weight (b w) per body surface (b s) and per energy content of the diet (1000 kcal)*

Age	Sex	Zinc intakes mg/day	mg/kg b w	mg/m b s	mg/1000 kcal	Remarks	Ref
6-8 days		0 7 5 0	0 2–1 2			Breastmilk	(4)
1 5 weeks		1 0 1 5				Breastmilk cow s milk	(1)
1–3 years		4 6	0 537		2 87	Children s home	(25)
3 5 years		5–7	0 3–0 4	8–10	4–5	Mixed diet	—
3–7 years		7 1	0 537		2 78	Children s home	(25)
7 9 years	f	4 7–6 9				Balance studies	(16)
8 1 years		16	0 5			Balance studies	(11)
7 16 years		13 6	0 537		4 31	Children s home	(25)
10–13 years		9–13	0 2–0 4	7–11	4–5	Mixed diet	—
10–12 years					5 3	School feeding	(13)
14–30 years	f	1–20 (13)			9	Self selected diet	(27)
17–27 years	f	12				Balance studies	(2?)
Adults	m	14 4				Self selected diet	(21)
Adults		6–40					(26)
Adults		10–15					(8)
Households					2 6–3 0	Calculations	(6)

pidly after the newborn period Nevertheless the intakes and excretions become more or *less balanced (1)*

Preschool children (3–7 years) are reported by Vorob eva & Bol sanina (25) to have an average intake of 7 1 mg zinc per day This is about the upper range of the findings re ported here Related to body weights our find ings are much lower (Table 3)

For children aged 7 to 16 years data on zi c intakes are mainly reported from balance studies including a certain dietary regime (11 16) Consequently those data are not con pletely comparable to our results obtained un der *ad libitum* feeding Our findings are lower than those of Macy (11) but higher than those of Price (16) (Table 3) The zinc intakes re ported by Vorob eva & Bol sanina (25) for this age group are in the upper range of our findings and exceed ours if expressed per body weight

Zinc intakes of adults are reported mainly around 10–15 mg/day (Table 3) This cor responds to about 0 2 mg per kg body weight and is the same as the lower data of the school children reported here

The availability of zinc in the diet is in fluenced by a variety of factors (23) Based on balance studies of McCance & Widdowson (12) daily intakes of 10–15 mg zinc per day

Table 4 *Data on zinc balances and recommended intakes per day*

Age group	Sex	Balance Studies: Zinc intakes mg	Zinc intakes mg/kg b w	Zinc retentions mg	Zinc retentions mg/kg b w	Recommended intakes mg	Recommended intakes mg/kg b w	References
6-8 d			0.20–1 19		−0 85–+0 08			
3–6 y	m	3 8–5 9	0.22–0 31					(4)
7 10 y	f	4 6–9 3		−2 7–+3 4	−0 16–+2 0		0 307	(19)
8-1 y		15 ?–16 3	0 4–0 6			6		(7) (9) (14)
17–27 y	f	9 8–14 4		4 8	0 11			(11) (20)
Adults		5 6–22 0		5 1–8 8				(22)
Adults				−0 8–+2.7				(12)
—						10–15		(14)

Table 1 *Data on the children participating in the study*

Child	Sex	Age[a] (years)	Weight[a] (kg)	Height[a] (cm)	Surface[a b] (m²)	Number of weeks	Number of days
D S	m	2 81	17 5	97 2	0 6695	2	13
J R	f	3 16	14 6	96 4	0 616	6	34
S S	f	4 11	16 2	107 2	0 684	1	7
G K	m	4 52	19 5	113 2	0 783	2	14
S C	f	4 95	18 7	110 3	0 755	1	5
T G	f	5 06	18 7	113 2	0 769	1	7
J S	f	9 67	42 5	139 6	1 269	1	7
S M	f	11 28	32 6	146 3	1 172	3	18
A R	f	11 80	33 2	138 2	1 134	1	4
R R	f	12 75	41 5	156 0	1 362	5	33
O M	m	13 42	33 4	137 7	1 134	1	7
Total 11						24	149

[a] Average for the period studied
[b] Body surface (cm²) = weight 0 425 × height 0 725 × 71 84 (3)

pensated for by averaging over a period of one week only

The average zinc intakes of the different children and of the two age groups are related to body weights body surfaces and to the energy contents of the daily food intakes The children had between 0 2 and 0 4 mg zinc per kg body weight and day As with other nutrients we find a tendency of decreasing intakes per body weight with increasing age No such tendency can be seen with the intakes if expressed per body surface

The zinc contents of the diets ranged from 1 7 to 9 0 mg/1 000 kcal The averages for the different children varied between 3 9 and 5 2 mg zinc/1 000 kcal

DISCUSSION

With breastfed newborns intakes of 0 7 to 5 0 mg zinc per day are reported, resulting in negative balances (4) (Table 3) Due to the rapidly decreasing zinc content of breast milk the zinc intakes of infants are decreasing ra

Table 2 *Zinc intakes of children per day per calorie intake per weight and per body surface*

Child	Age (years)	Sex	Weeks	mg/day average	Zinc Intakes: Range of intakes mg/day: Per day	Average per week	mg/1000 kcal	mg/kg	mg/m²
D S	2 8	m	2	5 81	3 78– 7 76	5 70–5 92	3 98	0 332	8 67
J R	3 2	f	6	5 32	3 52–11 27	4 55–6 09	4 14	0 364	8 64
S S	4 1	f	1	7 09	4 54– 7 98	—	4 72	0 438	10 36
G K	4 5	m	2	7 42	5 09–11 08	6 08–8 76	4 23	0 380	9 48
S C	5 0	f	1	6 68	4 31– 9 59	—	4 04	0 357	8 84
T G	5 1	f	1	5 81	3 94– 7 40	—	4 07	0 311	7 55
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Table 5 *Relations between zinc and main constituents of the diets*

Correlation coefficient	Energy	Protein	Carbohydrates	Fat	Dry matter
r	0 6713	0 7632	0 5699	0 2206	0 6726

have been recommended for adults (14) (Table 4), 6 mg zinc per day is supposed to be adequate for young school children Compared with the data summarized in Table 4 the intakes of zinc reported here may be regarded adequate

The zinc content of the daily food intakes related to the energy content is similar to the findings in US school lunches (13) but far below those reported by White (27) (Table 3) and higher than those reported for infants and preschool children (25) Droste & Jekat (6) calculated the zinc content of an average diet of families in Germany from 1950–1962 The results are considerably lower than our data probably due to still missing data for the zinc content of some foodstuffs

Zinc contents of the diets were correlated to their contents of energy protein carbohydrate, fat and dry matter (Table 5) The highest correlation exists between protein and zinc, the lowest between fat and zinc Similarly Murphy et al (13) found a comparatively high correlation between zinc and protein in US school lunches as opposed to the relation between zinc and energy content The close relation between zinc and protein is confirmed by a survey of the zinc contents of different foodstuffs (18) Foods containing more than 10 ppm zinc are mainly those with a high protein content From this one may assume that zinc deficiency is a contributing factor to signs of protein deficiency

SUMMARY

During one full year the zinc contents of 149 daily food intakes of 11 children were analysed The average content was 4–5 mg zinc per 1 000 kcal Zinc was mainly related to the protein contents of the diets Children aged 3–5 years had an intake of 5–7 mg zinc per day, children aged 10–13 years had 9–13 mg zinc per day Related to body weight the intakes were 0 2–0 4 mg/kg slightly decreasing with increasing age Related to the body surface the intakes were 7–11 mg/m, independent of age The results are discussed in the context of other findings Compared with balance studies, the intakes may be regarded adequate

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TRANSILLUMINATION OF THE SKULL IN INFANTS AND CHILDREN

Recording with a New Point Scale

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From the Department of Paediatrics (Head Professor Stig Sjölin) University Hospital Uppsala Sweden and the Ethiopian Nutrition Institute (Head Dr Bo Åkerrén) Addis Ababa Ethiopia

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THE MAIN USE AND LIMITATIONS OF TRANSILLUMINATION

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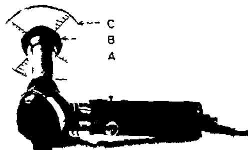


Fig 1 An ordinary transillumination lamp (Oculus) (*A*) with a black rubber adapter (*B*) fixed to the rubber rim held against the baby's head to which rim is attached a circular scale plate (*C*)

(6 7) Neither deep xanthochromia a protein content of up to 2 9 g per 100 ml (6) nor a cell count of up to 3 700 per mm³ (11) prevent transillumination of the cerebrospinal fluid

The aim of this paper is to draw further attention to the very useful and easily performed transillumination examination as a routine procedure in infancy and to present a simple method of transillumination with the use of a standardized scale plate by which it is possible to roughly objectivize the results within different scale points

Some findings in different groups of infants and children in Ethiopia will be presented later (5)

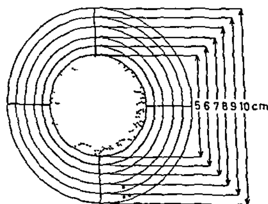


Fig 2 The scale plate its markings and measurements All the newborn infants in our test group illuminated frontotemporally the field marked with one point (1) and in no case was there illumination over the peripheral circular marking of this field *Parieto-occipitally all* the infants illuminated the central circular marking (0 5) as well as a few mm of the field marked point one (1) The upper normal range of transillumination findings according to Svenningsen (14 15) in normal newborn babies and infants is indicated by the gray field for the frontal region (up to scale point 2) and for the parietooccipitally region by the dotted field in the figure (up to scale point 1)

METHOD

In order to make it possible to objectivize the transillumination findings without complicating the method of examination we have constructed a transparent point scale which is attached to the transillumination lamp

The transillumination lamp used is of the Oculus type (Fig 1) with a small 25 watt flashlight

Fixed to the rim held against the infant's head is a black rubber adapter to which is attached a circular scale plate of plexiglas 10 cm in diameter In the centre of the scale plate is a hole to the inside of which the rubber adapter is attached and outside the hole there are concentric circles at distances of 0 5 cm (Fig 2) The plate is divided into four quadrants marked with a diminishing number of points from the periphery towards the centre 5 points in the periphery followed by 4 3 and 2 points and 1 point in the centre

The examinations were performed in a totally darkened room where the examiner had adapted his eyes to the darkness for 3-5 min As routine all infants were examined over the frontotemporal and parieto-occipital region on the right as well as the left side of the head

RESULTS AND DISKUSSION

Transillumination findings in normal infants and children using the lamp source and method described above

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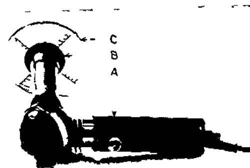


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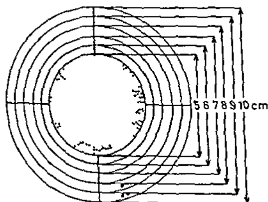


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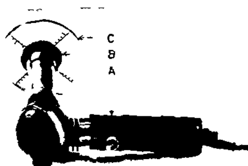


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The aim of this paper is to draw further attention to the very useful and easily performed transillumination examination as a routine procedure in infancy and to present a simple method of transillumination with the use of a standardized scale plate by which it is possible to roughly objectivize the results within different scale points

Some findings in different groups of infants and children in Ethiopia will be presented later (5)

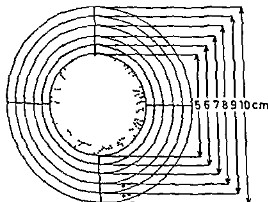


Fig 2 The scale plate its markings and measurements All the newborn infants in our test group illuminated frontotemporally the field marked with one point (1) and in no case was there illumination over the peripheral circular marking of this field Parieto-occipitally all the infants illuminated the central circular marking (0 5) as well as a few mm of the field marked point one (1) The upper normal range of transillumination findings according to Svenningsen (14 15) in normal newborn babies and infants is indicated by the gray field for the frontal region (up to scale point 2) and for the parietooccipitally region by the dotted field in the figure (up to scale point 1)

METHOD

In order to make it possible to objectivize the transillumination findings without complicating the method of examination we have constructed a transparent point scale which is attached to the transillumination lamp

The transillumination lamp used is of the Oculus type (Fig 1) with a small 25 watt flashlight

Fixed to the rim held against the infant s head is a black rubber adapter to which is attached a circular scale plate of plexiglas 10 cm in diameter In the centre of the scale plate is a hole to the inside of which the rubber adapter is attached and outside the hole there are concentric circles at distances of 0 5 cm (Fig 2) The plate is divided into four quadrants marked with a diminishing number of points from the periphery towards the centre 5 points in the periphery followed by 4 3 and 2 points and 1 point in the centre

The examinations were performed in a totally darkened room where the examiner had adapted his eyes to the darkness for 3–5 min As routine all infants were examined over the frontotemporal and parieto-occipital region on the right as well as the left side of the head

RESULTS AND DISKUSSION

Transillumination findings in normal infants and children using the lamp source and method described above

The scale plate was worked out after 80 examinations in healthy newborn fullterm infants None of the infants transilluminated with a light halo more than 6 cm in diameter which means at a maximum up to scale point 2

The normal range of transillumination findings according to Svenningsen (14 15) who has examined several hundreds of infants and children by using a transillumination lamp

Th new scale plate as well as the Oculoscope can be ordered from Kifa S-171 95 Solna Sweden

with a light source comparable with ours (15), is presented below and in Fig 2

Normal newborn infants transilluminate *frontally* with a light halo 5–6 cm in diameter, i e up to scale point 2 or less on our scale plate, *parieto occipitally* with a halo 4–5 cm in diameter, i e up to scale point 1 or less on our scale plate

Children of 12 months of age and more transilluminate with a light halo 4 cm in diameter or less which means that they should not illuminate the scale plate at all

SUMMARY

Transillumination should be used as a routine method of examination in all neurological disorders in infants It is harmless for the patient simple to perform gives qualitative information and may differentiate between subdural effusions, simple hydrocephalus cerebral malformations and cerebral atrophies

A new method of recording the transillumination findings with a point scale plate is presented by which it is possible to express the findings in objective figures without increasing the complexity of the method

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OSTEOPETROSIS ASSOCIATED WITH PROXIMAL AND DISTAL TUBULAR ACIDOSIS

M VAINSEL, P FONDU S CADRANEL, CL ROCMANS and W GEPTS

From the Departments of Paediatrics and Biochemistry University of Brussels Belgium

Recently we had the opportunity to observe a child exhibiting renal tubular acidosis (RTA) with generalized marble bone disease Data are presented here which demonstrate that the renal defect involved both the proximal and the distal tubules Neither classical studies nor metabolic investigations demonstrated a relationship between RTA and osteopetrosis

CASE REPORT

G S a male infant, was born in June 1964 after a full term uncomplicated pregnancy from parents who were first cousins His birth wei ht was 4 0 kg The first weeks after he was discharged from the hospital growth was poor blood examination revealed anemia at the age of 2 / months Bone marrow examination was normal and he was given iron therapy for a few weeks Afterwards he did well growth followed the tenth percentile and psycho-motor development was normal At the age of two an X ray taken after a fall disclosed osteopetrotic bones Similar investigations of the parents and of the two other children did not show any abnormality From the age of three th child experienced hort periods of unexplained apathy At the age of four laboratory data showed values of acid base equilibrium consistent with strong metabolic acidosis and the child was admitted to the University Pediatric Center

Height was 97 cm and weight 15 3 kg (both just below the tenth percentile) Physical examination showed a moderate thoracic deformity and a marked bilateral genu valgum Neither hepatosplenomegaly nor abnormal neurological signs were present. Psychomotor development was normal Laboratory data were as follow pH 7 14 7 09 7 17 total blood CO content (t CO) 15 9 10 2 14 7 mmol/l creatinine 0 73 mg 100 ml urea 34 mg/100 ml Na 131 mEq/l K 3 6 mEq l Cl 114 mEq/l P 6 8 mg/100 ml Ca 9 3 mg/100 ml Urinary pH was markedly high (6 50 to 7 10) There was slight proteinuria Hematological data were hemoglobin 11 2 g/100 ml WBC 7 800 with normal differential count erythrocyte count 3 800 000 per cubic millimeter X ray showed generalized osteopetrosis (Fig 1) Intravenous pyelography was normal there was no evidence of nephrocalcinosis

After the first metabolic studies the child was started on citrate therapy No improvement was observed for several months He was then readmitted at the age of five for further investigations He had grown 9 cm in 12 months remaining in the 10th percentile Physical examination disclosed pallor and hepatosplenomegaly (both the liver and the spleen were 3 cm below th costal margin) Laboratory data were as follow pH 7 11 t CO 8 2 mmol/l P 6 3 mg/100 ml Ca 8 9 mg/100 ml Na 142 mEq/l K 3 mEq/l Cl 119 mEq/l Erythrocyte count was 3 750 000 per cubic millimeter hemoglobin 10 75 g/100 ml and platelet count 230 000 Serum iron was 70 μg/100 ml and iron binding capacity 312 μg/100 ml Neither albumin nor reducing substances were found in urine Bone marrow was hypoplastic with a total nucleated cell count of 29 000 per cubic millimeter and normal average values of cellular elements

Metabolic studies

Net acid excretion was evaluated during spontaneous acidosis and after ammonium chloride loading (3) Bicarbonate excretion was studied during water diuresis (14) Determination of the renal bicarbonate threshold and bicarbonate Tm were performed using the procedure described by Edelman et al (2)

The reabsorptive capacity for phosphate was determined by calculation of maximal tubular reabsorption of phosphate (TmP) after an intravenous loading of phosphate (1)

Amino-acids were determined by column chromatography using the method of Moore Spackman & Stein (7)

For ^{59}Fe test, 15 μCi high activity ^{59}Fe were incubated with AB plasma and injected intravenously (11) For autologous erythrocyte ^{51}Cr t½ and blood volume measurements 14 ml autologous blood were

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Table 3 *Hydrogen ion excretion spontaneous before and after ammonium chloride administration*

	Blood tCO	Urine pH	Na	K+	Cl−	μEq/min/1 73 m T A[b]	NH +	HCO_3^-
I	9 3	6 95	179	71	175	3 2	11 5	31 6
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tCO total blood CO_2 content
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Consanguinity in the parents and the lack of other familial case drew attention to be pos sibility of a recessive mode of inheritance In this category malignant clinical features are often present (5 6)

This child had only a slight degree of anemia with hepatosplenomegaly The lack of in

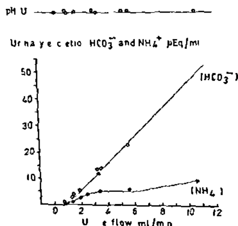


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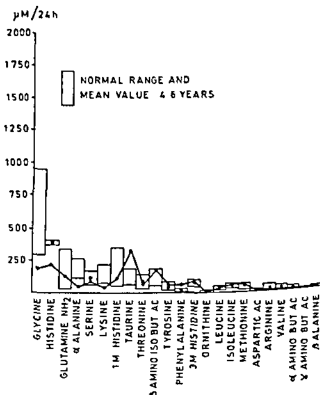


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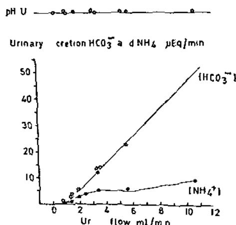


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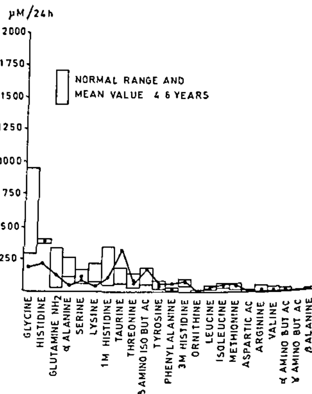


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	Blood tCO_2 [a]	Urine				μEq/min/1 73 m²		
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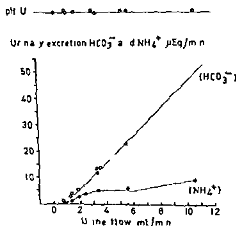


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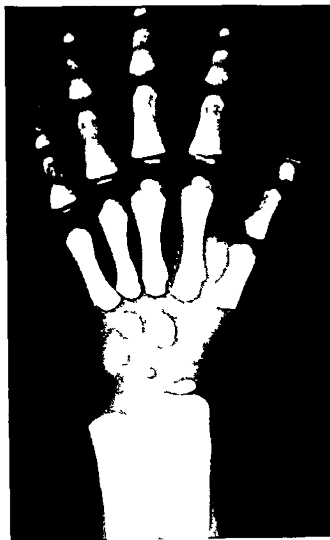


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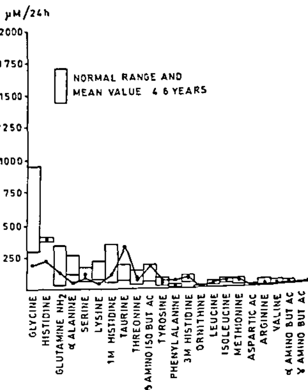


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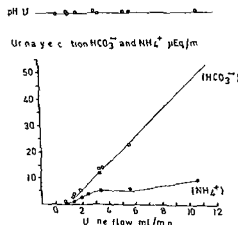


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Ammonium chloride loading During spontaneous acidosis urinary pH is high (Table 3) excretion of titratable acid and $NH4^+$ is low trivial amounts of bicarbonate are found There is no increased excretion of titratabl

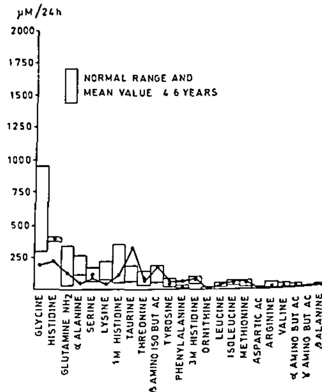


Fig 2 Chromatography of amino acids of deproteinised urine



Fig 7 Renal biopsy Flattened epithelial cells with thickened basement membrane in one convoluted tubule (H E. × 450)

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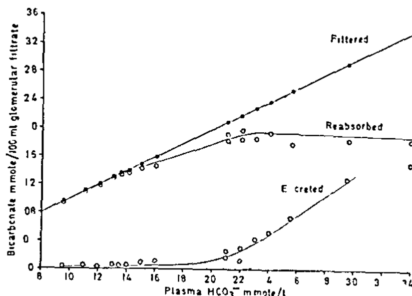


Fig 4 Curves of renal bicarbonate reabsorption and excretion durin bicarbonate infusion Interrupted lines represent normal values of reabsorbed bicarbonate for the upper line and excreted bicarbonate for the lower line

creased splenic sequestration of erythrocytes and of thrombopenia or neutropenia excluded hypersplenism External measurements also excluded extramedullary erythropoïesis The only explanation for the anemia was a very slight decrease in medullary erythropoïesis as assessed by measurement of ^{59}Fe activity over sacral marrow and by recovery of the administered dose of this isotope in the circulating red cells (71 %) Hematological findings were thus consistent with the assertion of a benign form of osteopetrosis (15) in spite of clinical findings and recessive mode of inheritance

Daily study of both blood and urinary pH led us to suspect a distal RTA In this disorder the distal nephron is not able to maintain a normal steep lumen peri tubular gradient (12 14) In our case net acid excretion during spontaneous acidosis was low when related to total blood CO_2 content (3) Ammonium chloride loading failed to significantly increase titratable acid and $NH4^+$ Furthermore the pattern of bicarbonate excretion during water excretion was similar to that found by Seldin & Wilson (14) in distal renal tubular acidosis However citrate therapy being unsuccessful the possibility of an associated proximal tubular defect was considered The renal bicarbonate threshold and $TmHCO_3^-$ were very low more than 25 % of the filtered bicarbonate load was excreted at normal plasma bicarbonate levels The classification of infantile RTA by Soriano et al (16) comprises two types a proximal defect with a low threshold and low or normal $TmHCO_3^-$ a distal defect with a normal threshold and normal $TmHCO_3^-$ Our results suggest a defect of the acidification process of both proximal and

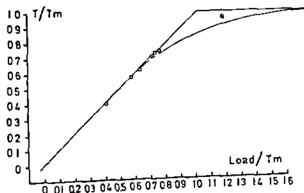


Fig 5 Splay of bicarbonate reabsorption curve

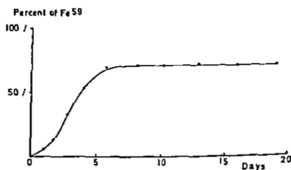


Fig 6 Red blood cells utilization of ^{59}Fe



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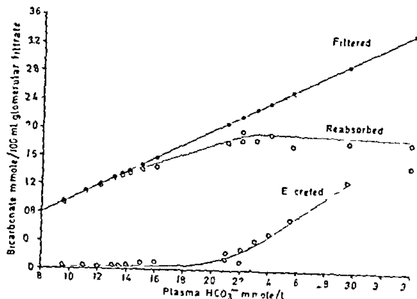


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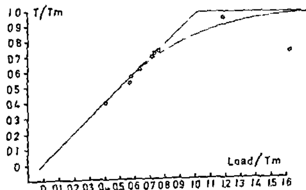


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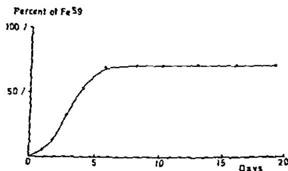


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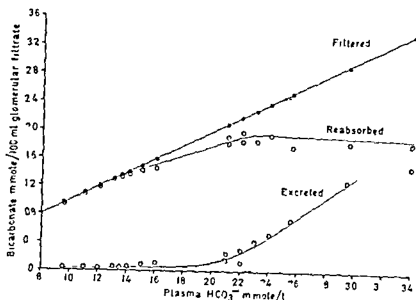


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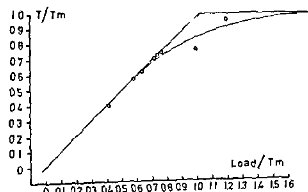


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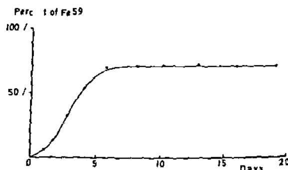


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Key words Osteopetrosis renal tubular acidosis anemia

Acta Pædiat Scand 61 435–441 1972

PLASMA GLUCAGON THYROTROPIN GROWTH HORMONE AND INSULIN RESPONSE TO COLD EXPOSURE IN THE HUMAN NEWBORN

M FEKETE, R D G MILNER GY SOLTÉSZ, R ASSAN and J MESTYAN

From the Department of Paediatrics University of Pecs Hungary Department of Child Health University of Manchester England and Faculte de Médecine de Paris Paris France

Cold exposure in the human newborn is associated with an increase in metabolic rate and oxygen consumption (3 17 20) Lipolysis in brown and white fat stimulated in part by catecholamines is probably an important source of substrate for the increased metabolism associated with cold exposure but the question of whether the metabolic changes due to cold should be ascribed solely to the release of lipid from brown fat by catecholamines remains unanswered Hull (13) observed that the thermogenic response of the newborn rabbit was not blocked by pronethalol The role of other hormones with a known lipolytic activity in neonatal thermogenesis such as glucagon growth hormone (GH) or thyrotropin (TSH) and of insulin which is antilipolytic has not hitherto been studied

The purpose of the present study was to investigate whether changes in the plasma concentrations of such hormones did occur in the human newborn when exposed to cold Plasma concentrations of glucose free fatty acids (FFA) lactate and α amino nitrogen were measured in addition to the hormones to determine what interrelationships if any existed between hormone and metabolite under the conditions of the investigation Three clinically defined groups of infants were studied full term normal full term small for dates and preterm The design of the study made it possible to compare also the levels of metabolites and hormones in the different clinical groups under well defined control conditions

MATERIAL

Communicated parental consent was obtained for the investigation of each infant The infants studied were divided into three groups In all cases the gestational age was estimated as the number of completed weeks from the first day of the last menstrual period and the size for gestational age was calculated by reference to local standards (6)

Full term normally grown

Nine infants of 37–42 weeks gestational age all of whom were above the 10th centile for birth weight Four were above the 90th centile but in none of these was there evidence of maternal diabetes Two pregnancies were complicated by mild pre-eclamptic toxaemia and one of these infants was delivered by caesarian section because of foetal asphyxia All other infants were normal vaginal deliveries and no clinical abnormality was detected postnatally

Full term small for dates

Ten infants of 38 42 weeks gestational age all of whom were below the 10th centile for weight All but 2 were normal vaginal deliveries one was delivered by forceps because of a breech presentation and one by caesarian section for maternal reasons No clinical abnormalities were detected postnatally

Preterm

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Twelve infants of 29–36 weeks gestational age of whom 2 were below th 10th centile and on was above the 90th centile for weight. One pregnancy was

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PLASMA GLUCAGON THYROTROPIN GROWTH HORMONE AND INSULIN RESPONSE TO COLD EXPOSURE IN THE HUMAN NEWBORN

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Cold exposure in the human newborn is associated with an increase in metabolic rate and oxygen consumption (3 17 20) Lipolysis in brown and white fat stimulated in part by catecholamines is probably an important source of substrate for the increased metabolism associated with cold exposure but the question of whether the metabolic changes due to cold should be ascribed solely to the release of lipid from brown fat by catecholamines remains unanswered Hull (13) observed that the thermogenic response of the newborn rabbit was not blocked by pronethalol The role of other hormones with a known lipolytic activity in neonatal thermogenesis such as glucagon growth hormone (GH) or thyrotropin (TSH) and of insulin which is antilipolytic has not hitherto been studied

The purpose of the present study was to investigate whether changes in the plasma concentrations of such hormones did occur in the human newborn when exposed to cold Plasma concentrations of glucose free fatty acids (FFA) lactate and α amino nitrogen were measured in addition to the hormones to determine what interrelationships if any existed between hormone and metabolite under the conditions of the investigation Three clinically defined groups of infants were studied full term normal full term small for dates and preterm The design of the study made it possible to compare also the levels of metabolites and hormones in the different clinical groups under well defined control conditions

MATERIAL

Communicated parental consent was obtained for th investigation of each infant The infants studied were divided into three groups In all cases the gestational age was estimated as the number of compl ted weeks from the first day of th last menstrual period and the size for gestational age was calcu ated by reference to local standards (6)

Full term normally grown

Nine infants of 37–42 weeks gestational age all of whom were above the 10th centile for birth weight Four were above the 90th centile but in none of these was there evid nce of maternal diabetes Two pregnancies were complicated by mild pre-eclampti toxaemia and one of these infants was delivered by caesarian section b cause of foetal asphyxia All oth r infants were normal vaginal deliveries and no clinical abnormality was detected postnatally

Full term small for dates

Ten infants of 38–42 weeks gestational age all of whom were below th 10th centile for weight All but 2 were normal vaginal deliveries one was delivered by forceps because of a breech presentation and one by caesarian section for maternal reasons No clinical abnormalities were detected postnatally

Preterm

Twelve infants of 29 36 weeks g stational a e of whom 2 were below th 10th centile and on was above the 90th centile for weight One pre_nancy was

Table 1 Sex, birth weight, gestational age and postnatal age of the three groups of newborns

Clinical description	n	Sex		Birth weight (g)		Gestational age (weeks)		Postnatal age (hours)	
		Male	Female	Mean	Range	Mean	Range	Mean	Range
Full term normal grown	9	4	5	3 555	2 750–4 530	38	37–42	46	11–72
Full term small for dates	10	6	4	2 468	2 200–2 800	39	38–42	49	24–72
Preterm	12	4	8	1 892	1 340–2 950	32	29–36	42	29–48

complicated by pre-eclampsia There were 3 abnormal deliveries 2 caesarian sections were performed for maternal reasons and one infant was a twin born by the breech One infant definitely had signs of neurological abnormality and doubtfully abnormal signs occurred in another

The clinical details and postnatal age at the time of study of the infants are presented in Table 1

METHODS

Each infant was examined 4-6 hours after the last meal when a catheter was inserted 4-6 cm into the umbilical vein The naked infant was placed in an incubator at 32-35 C Deep body temperature was measured by a thermocouple inserted 10-12 cm into the rectum After 30 min the infant had settled and oxygen consumption was measured continuously for a further 30 min with a Kipp-Noyon diaferometer (16) Immediately after the measurement of oxygen consumption was concluded a heparinised blood specimen was withdrawn from the umbilical vein for plasma metabolite and hormone determinations The naked infant was then exposed to room temperature (22 5-28 C) for a further hour and the same protocol was repeated The upper chest and head of the baby was covered by the perspex hood of the diaferometer and the temperature within the hood was 1 0 to 1 5 C higher than that outside In the warm incubator the infants became quiet a few minutes after the insertion of the catheter At room temperature they all became intermittently restless and had increased muscular activity including crying Twenty ml blood was the maximum amount removed from any infant No complication arose during or after the investigation

The blood samples were centrifuged within 2 hours and plasma aliquots were stored at −20 C until hormone assays could be performed Blood glucose was measured by the method of Pryce (21) plasma FFA according to a combination of the methods of Dalton & Kowalski (5) and Laurell & Tibling (15) plasma α amino nitrogen by the method of Rubinstein & Pryce (22) and blood lactate according to Barker & Summerson (2) as modified by Huckabee (12) Plasma insulin was measured by immunoassay (9) using insulin binding reagent (Wellcome Reagents Ltd) and a human insulin standard (66/304 kindly given by the Medical Research Council) Plasma GH was measured by immunoassay using growth hormone binding reagent (Wellcome Reagents Ltd Beckenham Kent) filtration for the separation of free and bound hormone and a human GH standard (66/217 kindly donated by the Medical Research Council) Plasma TSH was measured by Professor R Hall of the University of Newcastle by the immunoassay of Hall Amos & Ormston (10) using a human TSH standard (Medical Research Council Standard A) Plasma glucagon was measured by immunoassay (1) using a pork glucagon standard (kindly given by Dr Bouchet of Novo Paris) and an antibody to glucagon like immunoreactive material which cross reacted weakly with gut glucagon

RESULTS

Oxygen consumption and plasma metabolites and hormones at 32–35 C

There was no significant difference in the oxygen consumption of the three groups of infants in the warm environment (Table 2) A comparison of the plasma metabolite and hormone concentrations in the thermoneutral environment revealed no significant difference between the full term–normal and the full term–small for dates groups In the preterm infants the mean blood glucose and plasma insulin were lower and the plasma FFA and GH were higher than in either of the full term groups but because of the variation within each group only the difference in the mean blood glucose level was significant ($p < 0.02$)

Various tests of correlation were performed on the different measurements made in the warm environment An analysis of plasma hormone concentration against gestational age or postnatal age in each of the three groups

Table 2 *Rectal temperature oxygen consumption plasma metabolite and hormone concentrations in different groups of infants at 32–35 C*

	Full term normal (9)		Full term small for dates (10)		Premature (12)	
	Mean ± S E M	Range	Mean ± S E M	Range	Mean ± S E M	Range
Temperature & O_2 consumption						
Environmental temp (C)	33 2±0 3	32 0–34 0	33 9±0 1	32 5–34 5	34 0±0 2	33 0–35.5
Rectal temperature (C)	37 2±0 1	36 9–37 8	36 6±0 1	35 8 37 6	36 5±0 1	35 4–37 8
O_2 consumption (ml/kg min)	4 8±0 2	4 1– 6 5	4 5±0 2	3 6– 6 3	4 3±0 4	3 1– 6 5
Metabolites						
Glucose (mg/100 ml)	62±2	54–78	64±3	49–86	51±3*	26–72
FFA (μM)	838±128	390–1660	956±99	550–1660	1 097±170	560–2 000
Lactate (mg/100 ml)	13 7±2 2	7 0–29 0	13 5±1 2	9 0–21 0	14 4±2.0	7 0–33 0
α amino nitrogen (mg/100 ml)	4 2±0 3	3 3–4 9	3 8±0 3	2 2–4 7	4 1±0 2	2.7–6.2
Hormones						
Insulin (μU/ml)	15±2	11–36	14±3	5–33	11±1	7–19
Glucagon (pg/ml)	349±44	90–510	260±42	90–495	390±63	90–765
GH (ng/ml)	32±9	3–95	37±11	5–104	48±12	11–167
TSH (μU/ml)	10±2	3–18	10±1	5–16	11±4	3–47

Indicates a significant difference between the mean values for the preterm and for the full term normal or small for dates infants

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In the full term normal group there was no significant association between any metabolite and hormone In the full term small for dates infants blood glucose and plasma insulin were positively correlated ($p < 0 05$) In the preterm infants there was a negative correlation between blood glucose and plasma GH ($p < 0 05$) When the three groups were considered together a positive correlation between blood glucose and plasma insulin ($p < 0 05$) and a negative correlation between blood glucose and plasma GH ($p < 0 05$) were again observed

Rectal temperature oxygen consumption metabolite and hormone changes in response to cold exposure

In each of the three groups placing the baby in a lower temperature for 1 hour caused a fall in rectal temperature and a rise in oxygen consumption (Tables 3 4 and 5) The rise in oxygen consumption was similar in the different groups and the fall in rectal temperature was significantly greater in the preterm infants when compared with either of the full term groups No significant change was observed in any of the four metabolites in the full term normal group after 1 hour in a cold environment Similarly exposure to cold had no effect in any group on plasma α amino nitrogen levels In both the preterm and full term small for dates groups however cold exposure caused a significant rise in blood glucose and plasma lactate levels In the full term small

Table 1 *Sex birth weight, gestational age and postnatal age of the three groups of newborns*

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Table 2. *Rectal temperature oxygen consumption plasma metabolite and hormone concentrations in different groups of infants at 32–35°C*

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	Mean ± S E M	Range	Mean ± S E.M	Range	Mean ± S E M	Range
Temperature & O consumption						
Environmental temp (C)	33 2±0 3	32 0–34 0	33 9±0 1	32 5–34 5	34 0±0 2	33 0–35 5
Rectal temperature (C)	37.[illegible]±0 1	36 9–37 8	36 6±0 1	35 8–37 6	36 5±0 1	35 4–37 8
O consumption (ml kg min)	4 8±0 2	4 1– 6 5	4 5±0 2	3 6– 6 3	4 3±0 4	3 1– 6 5
Metabolites						
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Hormones						
Insulin (μU/ml)	15±2	11–36	14±3	5–33	11±1	7–19
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Table 3 *Metabolic and hormonal responses to cold in nine full term normal infants*

	In the cold		Cold–Warm
	Mean ± S E.M	Range	Mean ± S E.M
Temperature and O_2 consumption			
Environmental temperature (°C)	24 5±0 3	22 5–26 0	8 7±0 4***
Rectal temperature (°C)	36 6±0 1	35 9–37 3	0 5±0 1**
O_2 consumption (ml/kg/min)	8 0±0 4	5 1–9 9	3 2±0 4***
Metabolites			
Glucose (mg/100 ml)	62±2	53–79	0±1
FFA (μM)	958±201	440–2 500	120±102
Lactate (mg/100 ml)	17 3±2 6	9 5–34 0	3 6±2 1
α amino nitrogen (mg/100 ml)	4 0±0 3	2 8–5 5	−0 2±0 2
Hormones			
Insulin (μU/ml)	14±2	3–22	−1±2
Glucagon (pg/ml)	357±41	210–570	8±35
GH (ng/ml)	36±8	4–84	4±8
TSH (μU/ml)	7±1	1–15	−2±1

Level of statistical significance ** $p<0.01$ *** $p<0.001$

for dates group there was also a significant rise in plasma FFA The rises in FFA and lactate were appreciable but variable whereas the rise in glucose was slight but remarkably consistent

In none of the groups was there any significant change in the mean plasma concentration of insulin, glucagon, GH or TSH in response to cold exposure

DISCUSSION

This study was performed to investigate the possible role of hormones with known lipolytic and anti lipolytic actions in the metabolic responses of the human newborn to cold stress Interpretation of the results must be made bearing in mind certain features of the investigation

Since the cold exposure was defined by time

Table 4 *Metabolic and hormonal responses to cold in ten full term small for dates infants*

	In the cold		Cold–Warm
	Mean ± S E M	Range	Mean ± S E M
Temperature and O_2 consumption			
Environmental temperature (°C)	24 5±0 3	23 0–26 5	9 4±0 3***
Rectal temperature (°C)	35 8±0 2	33 8–36 7	0 7±0 1**
O_2 consumption (ml/kg/min)	8 1±0 8	3 4–12 4	3 5±0 6***
Metabolites			
Glucose (mg/100 ml)	69±4	52–97	5±1*
FFA (μM)	1 374±171	590–2 500	418±114**
Lactate (mg/100 ml)	21 6±2 1	13 0–34 0	8 1±1 8**
α amino nitrogen (mg/100 ml)	3 8±0 3	2 4–5 5	0 1±0 1
Hormones			
Insulin (μU/ml)	15±3	5–36	1±3
Glucagon (pg/ml)	289±34	90–405	29±34
GH (ng/ml)	33±6	7–65	−4±8
TSH (μU/ml)	11±2	5–22	0±1

Level of statistical significance * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Table 5 *Metabolic and hormonal responses to cold in twelve preterm infants*

	In the cold		Cold Warm
	Mean±S E M	Range	Mean±S E M
Temperature and O_2 consumption			
Environmental temperature (°C)	24 8±0 3	23 4–28 0	9 2±0 4* *
Rectal temperature (C)	35 2±0 2	33 9–37 1	1 3±0 1 **
O_2 consumption (ml/kg/min)	7 4±0 7	3 4–11 6	3 0±0 5
Metabolites			
Glucose (mg/100 ml)	58±3	40–82	7±2
FFA (μM)	1 380±196	340–2 500	283±150
Lactate (mg/100 ml)	26 0±5 4	9 1–76 0	11 5±3 4**
α amino nitrogen (mg/100 ml)	3 9±0 2	2.4–5 1	–0 2±0 2
Hormones			
Insulin (μU/ml)	13±1	2–24	1±1
Glucagon (pg/ml)	415±55	150 825	25±41
GH (ng/ml)	53±16	18–200	5±5
TSH (μU/ml)	14±5	3–64	2±1

Level of stati tical significance * $p<0\ 05$ * $p<0\ 01$ * * $p<0\ 001$

and environmental temperature it is probable that the premature infants were more stressed than either of the full term groups This con clusion is supported by the significantly greater fall in rectal temperature which occurred in the premature group The failure of cold to cause a change in any of the metabolites in the full term normal group in the present study may be taken as an indication of the moderate nature of the cold stress in this group It was of interest to observe that the stress so de fined which caused a similar rise in oxygen consumption in all three groups resulted in a significant rise in blood glucose and lactate in both the full term small for dates and the premature groups The possibility arises that the rise in plasma concentration of these metabolites represents a failure of normal homeostasis induced by the stress The results give no clue how this failure of homeostasis may occur In each group exposure to cold was accompanied by intermittent restlessness and increased muscular activity It was con cluded that the muscular activity made a con tribution to heat production and may have been responsible for the changes in plasma lactate levels In the overall metabolic response to cold the full term small for dates infants behaved more like the preterm than the full term normal infants

Plasma glucagon levels in the human new born have not previously been reported The results of the present study indicate that there is little difference in plasma levels of pan creatic glucagon sampled from the umbilical vein in each of the three clinical groups under control conditions and that no change in the mean plasma concentration occurs in response to cold exposure However Heding (11) has recently reported that glucagon is degraded rapidly in blood in vitro and the conditions of collection in the present study did not entirely exclude this possibility A small rise in plasma glucagon could have occurred which was mis sed by experimental artefact

Much attention has been paid to the effect of stress on plasma GH levels and the findings of Finkelstein et al (7) raise the possibility that the physiological plasma concentrations of GH in the newborn are much lower than those reported earlier (4 14 23) Care was taken to exclude stress other than cold from the present study The blood sampling had no haemodynamic effect on the infants as indi cated by a change in pulse rate and may be presumed not to have affected plasma GH

Table 3 *Metabolic and hormonal responses to cold in nine full term normal infants*

	In the cold		Cold–Warm
	Mean ± S E M	Range	Mean ± S E M
Temperature and O_2 consumption			
Environmental temperature (C)	24 5±0 3	22 5–26 0	8 7±0 4***
Rectal temperature (C)	36 6±0 1	35 9–37 3	0 5±0 1**
O_2 consumption (ml/kg/min)	8 0±0 4	5 1–9 9	3 2±0 4***
Metabolites			
Glucose (mg/100 ml)	62±2	53–79	0±1
FFA (μM)	958±201	440–2 500	120±102
Lactate (mg/100 ml)	17 3±2 6	9 5–34 0	3 6±2 1
α amino nitrogen (mg/100 ml)	4 0±0 3	2 8–5 5	−0 2±0 2
Hormones			
Insulin (μU/ml)	14±2	3–22	−1±2
Glucagon (pg/ml)	357±41	210–570	8±35
GH (ng/ml)	36±8	4–84	4±8
TSH (μU/ml)	7±1	1–15	−2±1

Level of statistical significance ** $p<0\ 01$ *** $p<0\ 001$

for dates group there was also a significant rise in plasma FFA The rises in FFA and lactate were appreciable but variable whereas the rise in glucose was slight but remarkably consistent

In none of the groups was there any significant change in the mean plasma concentration of insulin, glucagon GH or TSH in response to cold exposure

DISCUSSION

This study was performed to investigate the possible role of hormones with known lipolytic and anti lipolytic actions in the metabolic responses of the human newborn to cold stress Interpretation of the results must be made bearing in mind certain features of the investigation

Since the cold exposure was defined by time

Table 4 *Metabolic and hormonal responses to cold in ten full term small for dates infants*

	In the cold		Cold–Warm
	Mean ± S E M	Range	Mean ± S E M
Temperature and O_2 consumption			
Environmental temperature (C)	24 5±0 3	23 0–26 5	9 4±0 3***
Rectal temperature (C)	35 8±0 2	33 8–36 7	0 7±0 1**
O_2 consumption (ml/kg/min)	8 1±0 8	3 4–12 4	3 5±0 6***
Metabolites			
Glucose (mg/100 ml)	69±4	52–97	5±1*
FFA (μM)	1 374±171	590–2 500	418±114**
Lactate (mg/100 ml)	21 6±2 1	13 0–34 0	8 1±1 8**
α amino nitrogen (mg/100 ml)	3 8±0 3	2 4–5 5	0 1±0 1
Hormones			
Insulin (μU/ml)	15±3	5–36	1±3
Glucagon (pg/ml)	289±34	90–405	29±34
GH (ng/ml)	33±6	7–65	−4±8
TSH (μU/ml)	11±2	5–22	0±1

Level of statistical significance * $p<0\ 05$ ** $p<0\ 01$ *** $p<0\ 001$

Table 5 *Metabolic and hormonal responses to cold in twelve preterm infants*

	In the cold		Cold–Warm
	Mean ± S E M	Range	Mean ± S E.M
Temperature and O_2 consumption			
Environmental temperature (C)	24 8 ± 0 3	23 4–28 0	9 2 ± 0 4 **
Rectal temperature (C)	35 2 ± 0 2	33 9–37 1	1 3 ± 0 1**
O consumption (ml/kg/min)	7 4 ± 0 7	3 4–11 6	3 0 ± 0 5
Metabolites			
Glucose (mg/100 ml)	58 ± 3	40–82	7 ± 2*
FFA (μM)	1 380 ± 196	340–2 500	283 ± 150
Lactate (mg/100 ml)	26 0 ± 5 4	9 1–76 0	11.5 ± 3 4**
α amino nitrogen (mg/100 ml)	3 9 ± 0 2	2 4–5 1	−0 2 ± 0 2
Hormones			
Insulin (μU/ml)	13 ± 1	2–24	1 ± 1
Glucagon (pg/ml)	415 ± 55	150–825	25 ± 41
GH (ng/ml)	53 ± 16	18–200	5 ± 5
TSH (μU/ml)	14 ± 5	3–64	2 ± 1

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and environmental temperature it is probable that the premature infants were more stressed than either of the full term groups This conclusion is supported by the significantly greater fall in rectal temperature which occurred in the premature group The failure of cold to cause a change in any of the metabolites in the full term normal group in the present study may be taken as an indication of the moderate nature of the cold stress in this group It was of interest to observe that the stress so defined which caused a similar rise in oxygen consumption in all three groups resulted in a significant rise in blood glucose and lactate in both the full term small for dates and the premature groups The possibility arises that the rise in plasma concentration of these metabolites represents a failure of normal homeostasis induced by the stress The results give no clue how this failure of homeostasis may occur In each group exposure to cold was accompanied by intermittent restlessness and increased muscular activity It was concluded that the muscular activity made a contribution to heat production and may have been responsible for the changes in plasma lactate levels In the overall metabolic response to cold the full term small for dates infants behaved more like the preterm than the full term normal infants

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concentrations per se There is no ready explanation for the failure of cold to cause a change in plasma GH levels other than the wide range of levels observed in the thermo neutral environment

Fisher & Odell (8) reported that exposure of normal full term infants aged 3 hours to cold caused a significant rise in umbilical venous plasma TSH levels at 30 or 60 min The failure to demonstrate a similar rise in the present study may be due to the fact that the infants were aged 1 to 3 days If this interpretation is correct the response of the 1–3-day old in fant resembles that of the adult to cold more than that of the infant aged 3 hours The adult does not respond to cold stress with an increase in plasma TSH levels (19)

Plasma insulin levels were low in comparison to those previously reported from umbilical venous sampling in the newborn (18) and probably reflect basal pancreatic insulin release in the fasting newborn infant If this is correct it is not surprising that no further fall in the mean plasma level of insulin was seen on cold exposure

The failure of the present study to demon strate changes in plasma insulin glucagon GH or TSH to a cold stress may be due in part to the design of the study It seems more likely however that if these hormones do play a part in thermogenesis it is a relatively incon spicuous role in comparison to that of catecholamine release at the adipocyte

SUMMARY

Oxygen consumption, rectal temperature plasma metabolite and hormone concentrations were measured in 9 full term normal 10 full term, small for dates, and 12 preterm infants aged 1 to 3 days in a thermoneutral environment and in a cool environment At 32–34°C the oxygen consumption of all three groups was similar and the mean plasma metabolite and hormone concentrations of the full term normal and full term, small for dates groups were similar The mean blood glucose concentration of the preterm infants was significantly lower than that of the full term normal or full term small for dates group Considering the three groups together there was a significant positive correlation between blood glucose and plasma insulin and a signifi cant negative correlation between blood glu cose and plasma growth hormone concentra tions Placing the naked infants in an environ mental temperature of 22 5–28 0 C for 1 hour caused increased intermittent muscular ac tivity a significant fall in rectal temperature and a rise in oxygen consumption in each of the three groups There was no significant difference between the groups in the mean rise in oxygen consumption whereas the mean fall in rectal temperature in the premature group was significantly greater than that in either of the full term groups In the preterm group and the full term, small for dates group exposure to cold was associated with a signifi cant rise in the mean blood glucose and plasma lactate whereas no significant change occurred in any metabolite concentration in the full term normal group or in the plasma α amino nitrogen in any group In no group was ex posure to cold associated with a significant change in the mean plasma concentration of insulin glucagon growth hormone or thyro tropin

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SERUM MAGNESIUM LEVELS IN CHILDREN WITH CIRRHOSIS

GULTEN KAYA and ŞINASI ÖZSOYLU

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Magnesium (Mg^{++}) is the fourth most common cation in the body and it is second in abundance to potassium as an intracellular cation (11) Most of the intracellular Mg^{++} in soft tissues in the liver muscles brain and erythrocytes

Hypomagnesemia has been described in association with liver diseases in adults and in children (3, 7 11, 12) The pathogenesis of low serum magnesium concentration in patients with cirrhosis is not well understood Among other explanations, secondary hyperaldosteronism has been suggested as this increases excretion of Mg^{++} in the urine (3)

In order to clarify the findings of hypomagnesemia and the above possibility we have determined daily urinary excretion and serum Mg levels in 15 children with cirrhosis and in 15 age matched controls In addition, serum magnesium levels were determined in 10 children with hepatitis

MATERIALS AND METHODS

The diagnosis of cirrhosis was verified by the needle biopsy in all cases The ages of these patients ranged from 3 to 15 years With the exception of one all were boys

The patients were not given any medication or diuretics during the period of study Viral hepatitis was diagnosed clinically which was supported by the laboratory findings in 5 boys and 5 girls aged from 1 5 to 13 years The children in the control group did not have any history or clinical findings to suggest liver disease and the liver function tests were normal One of them had mild hemophilia but had not been transfused previously and 2 had upper respiratory tract infection

Urine and serum magnesium and creatinine in urine were determined using titan yellow (6) and modified Follin Wu (4) methods respectively

RESULTS

Serum Mg was found to be decreased in 7 (below the 2 standard deviation in 4) patients with cirrhosis and within normal limits in eight When the Mg^{++} values of the patients were compared with the controls it was found significantly decreased ($p < 0\ 05$) (Table 1) However, when 24 hourly urinary Mg^{++} excretions were compared no significant difference was found ($p > 0\ 50$) Urinary creatinine was determined so that we could be sure about the 24 hourly collections

Although the serum Mg level was increased in some of the patients with hepatitis this was not statistically significant ($p > 0\ 05$) (Table 2)

COMMENT

This report confirms the previous findings of hypomagnesemia in adults and in children with cirrhosis (3 7 11, 12) The mechanism for low plasma magnesium concentration in cirrhotic individuals may be due to several factors Stutzman & Amatuzio (10) proposed that the hypomagnesemia in this disease may be due to chronic malnutrition which is one of the known causes of hypomagnesemia (1, 2) Low levels of magnesium in cirrhosis may also be related to hypoalbuminemia (5) It has been reported that aldosterone increases the renal

Table 1 *Urinary excretion and serum values of Mg^{++} in children with cirrhosis and control cases*

Ages	Mg in serum mg/100 ml	Mg in urine (mg/24 hrs)	Amt of urine (ml/24 hrs)	Biopsy Remarks
4 5	1 8	61 6	700	Cirrhosis
6	1 93	40 77	750	Cirrhosis
6	2 44	69 28	500	Cirrhosis
8	1 63	18 32	850	Cirrhosis
12	1 93	41 48	850	Cirrhosis
4 5	2 6	46 22	550	Post necrotic cirrhosis
5	2 6	69 28	800	Post necrotic cirrhosis
6	1 63	55 5	550	Post necrotic cirrhosis
7	2 4	88 9	700	Post necrotic cirrhosis
9	1 66	36 65	550	Post necrotic cirrhosis
14	1 08	82 6	1 000	Post necrotic cirrhosis
12	1 7	57 60	600	Portal cirrhosis
13	1 7	39 61	700	Portal cirrhosis
14	1 42	70 35	700	Biliary cirrhosis
3	2 4	25 80	450	Cirrhosis & nephrotic syn
Patients	1 93 (S D 0 46)	53 59 (S D 25 77)	680 (S D 155 7)	
Controls	2 14 (S D 0 24)	62 46 (S D 25 77)	766 (S D 223)	
P	0 05 >	0 05 <	0 05 <	

excretion of magnesium (8) and the patients with cirrhosis have hyperaldosteronism Mal absorption resulting from portal hypertension and liver cirrhosis may be another cause of hypomagnesemia (9) Since the liver is the richest soft tissue source of magnesium hypo magnesemia may also be related to the fibrosis of this source (13) If the hypomagnesemia in liver cirrhosis was due to *urinary* excretion as a result of hyperaldosteronism the urinary ex cretion of this cation would be expected to be increased Although this was not a balance study increased urinary excretion was not confirmed by us Therefore more work is necessary in order to explain the causes of hypomagnesemia in this condition

However it should be stressed that the serum magnesium does not necessarily reflect total body magnesium Serum magnesium may be normal in the presence of severe depletion of total body stores or low with normal body stores

Serum magnesium levels of patients with viral hepatitis was determined in the early days of the disease and were slightly increased in four patients although statistically there was not any difference between these values and those of the control cases

Table 2 *Serum Mg^{++} levels of patients with viral hepatitis*

Age	Mg in serum (mg/100 ml)
2 5	2 5
4	2 5
1 5	2 4
2	2 75
7	2
4	2
10	1 88
5	2
5	1 95
13	2 7
Patients	[illegible]6 (S D 0 33)
Controls	2 14 (S D 0 24)
P	> 0 05

SUMMARY

Serum magnesium levels of 15 children with cirrhosis were determined and compared with the serum magnesium level of 15 age matched control children The diagnosis was verified in each case by liver biopsy The mean value was 2 14 (S D 0 24)/100 ml serum of the control and 1 93 (S D 0 46)/100 ml serum of the pa tients The difference between these values was statistically significant ($p < 0\ 05$) But there

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However it should be stressed that the serum magnesium does not necessarily reflect total body magnesium Serum magnesium may be normal in the presence of severe depletion of total body stores or low with normal body stores

Serum magnesium levels of patients with viral hepatitis was determined in the early days of the disease and were slightly increased in four patients although statistically there was not any difference between these values and those of the control cases

Table 2 *Serum Mg++ levels of patients with viral hepatitis*

Age	Mg in serum (mg/100 ml)
2 5	5
4	2 5
1 5	4
2	2 75
	2
4	2
10	1 88
5	2
5	1 95
13	2 7
Patients	2 76 (S D 0 33)
Controls	2 14 (S D 0 24)
P	> 0 05

SUMMARY

Serum magnesium levels of 15 children with cirrhosis were determined and compared with the serum magnesium level of 15 age matched control children The diagnosis was verified in each case by liver biopsy The mean value was 2 14 (S D 0 24)/100 ml serum of the control and 1 93 (S D 0 46)/100 ml serum of the pa tients The difference between these values was statistically significant ($p < 0\ 05$) But there

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SERUM MAGNESIUM LEVELS IN CHILDREN WITH CIRRHOSIS

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Magnesium (Mg^{++}) is the fourth most common cation in the body and it is second in abundance to potassium as an intracellular cation (11) Most of the intracellular Mg^{++} in soft tissues in the liver muscles brain and erythrocytes

Hypomagnesemia has been described in association with liver diseases in adults and in children (3 7 11, 12) The pathogenesis of low serum magnesium concentration in patients with cirrhosis is not well understood Among other explanations secondary hyperaldosteronism has been suggested as this increases excretion of Mg^{++} in the urine (3)

In order to clarify the findings of hypomagnesemia and the above possibility we have determined daily urinary excretion and serum Mg levels in 15 children with cirrhosis and in 15 age matched controls In addition serum magnesium levels were determined in 10 children with hepatitis

MATERIALS AND METHODS

The diagnosis of cirrhosis was verified by the needle biopsy in all cases The ages of these patients ranged from 3 to 15 years With the exception of one all were boys

The patients were not given any medication or diuretics during the period of study Viral hepatitis was diagnosed clinically which was supported by the laboratory findings in 5 boys and 5 girls, aged from 1 5 to 13 years The children in the control group did not have any history or clinical findings to suggest liver disease and the liver function tests were normal One of them had mild hemophilia but had not been transfused previously and 2 had upper respiratory tract infection

Urine and serum magnesium and creatinine in urine were determined using titan yellow (6) and modified Follin Wu (4) methods respectively

RESULTS

Serum Mg was found to be decreased in 7 (below the 2 standard deviation in 4) patients with cirrhosis and within normal limits in eight When the Mg^{++} values of the patients were compared with the controls it was found significantly decreased ($p < 0\ 05$) (Table 1) However when 24 hourly urinary Mg^{++} excretions were compared, no significant difference was found ($p > 0\ 50$) Urinary creatinine was determined so that we could be sure about the 24 hourly collections

Although the serum Mg level was increased in some of the patients with hepatitis this was not statistically significant ($p > 0\ 05$) (Table 2)

COMMENT

This report confirms the previous findings of hypomagnesemia in adults and in children with cirrhosis (3 7 11 12) The mechanism for low plasma magnesium concentration in cirrhotic individuals may be due to several factors Stutzman & Amatuzio (10) proposed that the hypomagnesemia in this disease may be due to chronic malnutrition which is one of the known causes of hypomagnesemia (1 2) Low levels of magnesium in cirrhosis may also be related to hypoalbuminemia (5) It has been reported that aldosterone increases the renal

Table 1 *Urinary excretion and serum values of Mg^{++} in children with cirrhosis and control cases*

Ages	Mg in serum mg/100 ml	Mg in urine (mg/24 hrs)	Amt of urine (ml/24 hrs)	Biopsy Remarks
4 5	1 8	61 6	700	Cirrhosis
6	1 93	40 77	750	Cirrhosis
6	2 44	69 28	500	Cirrhosis
8	1 63	18 32	850	Cirrhosis
12	1 93	41 48	850	Cirrhosis
4 5	2 6	46 22	550	Post necrotic cirrhosis
5	2 6	69 28	800	Post necrotic cirrhosis
6	1 63	55 5	550	Post necrotic cirrhosis
7	2 4	88 9	700	Post necrotic cirrhosis
9	1 66	36 65	550	Post necrotic cirrhosis
14	1 08	82.6	1 000	Post necrotic cirrhosis
12	1 7	57 60	600	Portal cirrhosis
13	1 7	39 61	700	Portal cirrhosis
14	1 42	70 35	700	Biliary cirrhosis
3	2 4	25 80	450	Cirrhosis & nephrotic syn
Patients	1 93 (S D 0 46)	53 59 (S D 25 77)	680 (S D 155 7)	
Controls	2 14 (S D 0 4)	62 46 (S D 25 77)	766 (S D 223)	
P	0 05 >	0 05 <	0 05 <	

excretion of magnesium (8) and the patients with cirrhosis have hyperaldosteronism Mal absorption resulting from portal hypertension and liver cirrhosis may be another cause of hypomagnesemia (9) Since the liver is the richest soft tissue source of magnesium hypo magnesemia may also be related to the fibrosis of this source (13) If the hypomagnesemia in liver cirrhosis was due to urinary excretion as a result of hyperaldosteronism the urinary ex cretion of this cation would be expected to be increased Although this was not a balance study increased urinary excretion was not confirmed by us Therefore more work is necessary in order to explain the causes of hypomagnesemia in this condition

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4	2
10	1 88
5	2
5	1 95
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Patients	2 76 (S D 0 33)
Controls	2 14 (S D 0 24)
P	> 0 05

SUMMARY

Serum magnesium levels of 15 children with cirrhosis were determined and compared with the serum magnesium level of 15 age matched control children The diagnosis was verified in each case by liver biopsy The mean value was 2 14 (S D 0 24)/100 ml serum of the control and 1 93 (S D 0 46)/100 ml serum of the pa tients The difference between these values was statistically significant ($p < 0\ 05$) But there

was no significant difference in the 24 hours urinary magnesium excretion between the two groups

Serum magnesium levels were determined in 10 children with viral hepatitis and compared with the controls No significant difference was found between these groups

ACKNOWLEDGMENT

Our sincere thanks are expressed to Professor A Kutsal and Mr H Kivanc of the Department of Statistics Hacettepe University for statistical analysis

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CYTOMEGALOVIRUS INFECTION AMONG INFANTS ADMITTED TO A PAEDIATRIC DEPARTMENT

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Infection with cytomegalovirus (CMV) is common in infants and children The infection can be demonstrated by isolation of virus from urine and sputum and by estimation of sero antibodies Both virus excretion and seroantibodies are known to persist for long periods of time after primary infection with CMV

Starr & Gold (16) have reported the finding of CMV uria at birth in 1 57% of 507 unselected neonates whereas Stern (17) in a study of 118 newborns and 136 hospitalized children aged 2 months to 4 years found CMV uria in 3% and 10% respectively

Complement fixing (CF) antibodies are found with quite varying frequency in children from the age of ½ to 2–5 years thus by Stern & Elek (18) and Hanshaw (10) in about 4 by Rowe et al (15) Carlstrom (6) Vaczi et al (19) and Baerlocher et al (5) in about 20 and by Chiba et al (8) in about 60 The relatively higher frequency of seropositivity among infants less than 6 months of age has been ascribed to the presence of maternal antibodies during the first months of life by the above mentioned authors

This report concerns the results of a study of CMV infections in infants less than 1 year of age admitted to a paediatric department during the course of 12 months

MATERIAL AND METHODS

Material

The study was undertaken amon all infants under 1 year of age admitted during the period September 20 1967 to September 19 1968 to the University Clinic of Paediatrics, Aarhus Kommunehospital Of the 357 infants admitted during that period about half were from Aarhus (population approximately 200 000 inhabitants) The rest were from smaller towns in Jutland or from rural areas Serological investigations were performed in 310 infants and in 251 of their mothers Isolation of CMV from the urine was attempted in 262 infants and in 8 mothers of infants excreting virus The blood and urine specimens were obtained during the first days after admission occasionally later In some cases attempts at isolation were also done using throat swabs The distribution by age and clinical diagnosis of the series is seen in Table 1

Methods

The methods used have previously been described in detail (4) and the following is therefore only a summary description Attempts at isolation of CMV from the urine and throat swabs were carried out using human embryonic lung tissue cultures in test tubes or small flasks The urine was collected in plastic bags and either immediately or at least within 3 hours inoculated onto the cultures Throat swabs were immersed in 1 ml culture medium which then served as the inoculum The strain of CMV isolated was carried through a few passages All strains were characterized by slowly developing focal cytopathogenic effects in tissue culture and typical intranuclear and cytoplasmic inclusions were seen in coverglass cultures stained with haematoxylin eosin

During the initial part of the study isolates from 7 infants were identified serologically as CMV by means of paired negative and positive human sera in an indirect immunofluorescent test as described earlier (4) Later isolates were frozen and stored at −70 C and then tested in a plaque neutralization test against a CMV hyperimmune serum made in rabbits with CMV laboratory strain Ad 169 (2) This was done with isolates from an additional 12 infants As all these isolates could be neutralized by the rabbit hyperimmune serum they were also classified as

Table 1 *Distribution according to age and diagnosis and the results of attempts at virus isolation on admission in 262 of 357 infants*

Clinical diagnosis		Age of infants <2 mo [a]	2–5 mo	6–12 mo	Total 0–12 mo
Respiratory infections	admitted	11	17	33	61
	studied	8	13	25	46
	CMV uria	0	2	3	5
Neurological disease	admitted	15	10	14	39
	studied	13	6	13	32
	CMV uria	0	0	1	1
Congenital heart disease	admitted	31	19	25	75
	studied	26	14	15	55
	CMV uria	1	0	0	1
Other conditions	admitted	87	53	42	182
	studied	58	43	28	129
	CMV uria	0	4	0	4
Total	admitted	144	99	114	357
	studied	105	76	81	262
	CMV uria	1 (1)	6 (8)	4 (5)	11 (4)

[a] 31 infants were newborn (< 7 days) 22 of these were investigated for CMV uria all negative

human CMV Isolates from the remaining two infants were not serologically identified because these viruses failed to grow after storage but on the basis of growth characteristics they were probably also CMV

The CF reaction was carried out with antigen produced from strain Ad 169 in a microtitre system (Cooke Engineering Co) using 4 units of antigen and 1 ¹/₂ units of complement Sera which did not fix complement in dilutions of 1 4 and 1 8 were considered negative Complement fixing sera were retitrated in two-fold dilutions from 1 2 to 1 256 and the titre expressed as the reciprocal value of the dilution giving a maximal haemolysis of 25 % All sera were re examined after up to 1 / years of storage at − 20 C against the same batch of antigen Paired sera from infants and sera from mother and infant were studied simultaneously Virus neutralizing (NT) antibodies were determined as described previously (1) using strain Ad 169

RESULTS

Virologic studies

On virus isolation attempts from urine performed on or shortly after admission CMV was isolated from 11 of 262 infants In infants less than 2 months of age viruria was diagnosed in 1 %, whereas 5–8 % of those studied in the age group 2–12 months had viruria (Table 1) An attempt at virus isolation was made again 2–13 months after the first virus isolation in 10 of the 11 infants with viruria It was found that all but one (case 9) still had viruria (Table 4)

In addition an attempt at virus isolation from the urine was also performed in 13 infants randomly selected among 21 infants sero positive on admission at an age of 6–12 months In these infants earlier attempts at isolation had been negative or had not been performed during admission In 10 of these 13 infants CMV could now be isolated (Table 5)

Thus a total of 11 + 10 infants were found in this study to have viruria These 21 infants constitute the clinical series of the present investigation (Table 4) At follow up all infants were tested for salivary excretion of CMV and CMV was isolated from the throats of 7 infants with viruria both at admission and at re examination but in none of the 10 infants in whom CMV-uria was detected only at the follow up study Virus was isolated from

the urine of only 1 of the 8 mothers studied (mother of case 12)

Serologic studies

A total of 33 ⁄ of the infants had CF anti bodies in their serum The frequency of sero positivity fell from 50% in infants less than one month of age to only 20% in infants aged 4–5 months A slight rise in the frequency of seropositive infants (to 24%) was seen in the 6–12 months age group (Table 2)

The high frequency of seropositivity among infants during the first months of life prompted a more detailed study of the role of maternal antibodies in the serologic picture employing sera collected simultaneously from 251 moth ers and their infants One hundred and fifty three mothers were seropositive and 98 nega tive Of the infants 81 (including one pair of twins) were seropositive and 171 were sero-negative Seventy eight of the seropositive infants had seropositive mothers whereas mothers to only 3 seropositive infants (includ ing the twins) were negative The age distribu tion of seropositive and negative infants from the seropositive mothers is given in Table 3 The frequency of positive infants from these mothers varied greatly in the different age groups—from 92% in infants less than 1 month of age to 18 ⁄ in infants 4–5 months of age whereas a higher frequency of seropositive infants (34) was found among the 6–12 month-old infants The mean levels of CF antibodies in the seropositive infants and their positive mothers are shown in the last co-lumns of the table The mean titre found in seropositive infants from the individual age groups was generally less than that in the mothers This was especially true in infants 1 and 2 months of age whereas the mean titre in infants less than 1 month old and their mothers and in the 6–12 month age group and their mothers was about the same level Using Mann Whitney s test the mean titre in the infants 1 month of age was found to be significantly lower (0 005 < 2α < 0 01) than the mean titre in infants less than 1

Table 2 *Results of serologic studies of infants on admission*

Age (months)	<1	1	2–3	4–5	6–12	Total
No positive	32	21	19	5	24	101
No studied	64	50	69	25	102	310
- positive	50	42	28	20	24	33

month of age Thus the fall in titre registered in the infants during the first 2 months of life was significant

The results of the serologic studies in in fants with viruria are shown in Table 4 All but one (case 9) were CF seropositive at the time of admission Case 9 developed CF anti bodies later but both sera tested contained NT antibodies (titre 40) At follow up 15 of 16 infants positive at admission still were seropositive whereas 1 (case 5) had become CF seronegative Significant changes in CF titre (≥4 fold rise or fall) were seen in only 7 patients All mothers to infants with viruria were themselves seropositive

In the age groups 6–12 months 24 infants were seropositive (Table 2) Virus isolation attempts have only been performed one or two times in 22 of these infants CMV being iso-lated from 13 (Table 4) Thus 60% of the sero positive infants in the age group 6–12 months were virus excreters

Table 3 *Serologic studies of mother infant couples with seropositive mothers*[a]

Infants age (months)	No posi tive	No stud ied	posi tive	Mean CF log titre ±1 S D: Positive infants	Mothers of positive infants
<1	24	26	92	3 6±1 3	4 3±1 7
1	14	18	78	2 4±1 0	4 8±1 0
2–3	15	28	54	2 8±1.2	4 9±1 6
4–5	3	17	18	2 7±2 1	4 3±0 6
6–12	22	64	34	3 6±1 4	4 6±1 3
Total	78	153	51		

Only 3 infants including 1 pair of twins from 98 sero-negative mothers were seropositive These infants were in the age groups <1 and 1 month when studied sero-logically and the titres were 2 8 and 8

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The high frequency of seropositivity among infants during the first months of life prompted a more detailed study of the role of maternal antibodies in the serologic picture employing sera collected simultaneously from 251 moth ers and their infants One hundred and fifty three mothers were seropositive and 98 nega tive Of the infants 81 (including one pair of twins) were seropositive and 171 were sero negative Seventy eight of the seropositive infants had seropositive mothers whereas mothers to only 3 seropositive infants (includ ing the twins) were negative The age distribu tion of seropositive and negative infants from the seropositive mothers is given in Table 3 The frequency of positive infants from these mothers varied greatly in the different age groups—from 92% in infants less than 1 month of age to 18 % in infants 4–5 months of age whereas a higher frequency of seropositive infants (34 %) was found among the 6–12 month old infants The mean levels of CF antibodies in the seropositive infants and their positive mothers are shown in the last co lumns of the table The mean titre found in seropositive infants from the individual age groups was generally less than that in the mothers This was especially true in infants 1 and 2 months of age whereas the mean titre in infants less than 1 month old and their mothers and in the 6–12 month age group and their mothers was about the same level Using Mann Whitney s test the mean titre in the infants 1 month of age was found to be significantly lower $(0\,005 < 2\alpha < 0\,01)$ than the mean titre in infants less than 1 month of age Thus the fall in titre registered in the infants during the first 2 months of life was significant

The results of the serologic studies in in fants with viruria are shown in Table 4 All but one (case 9) were CF seropositive at the time of admission Case 9 developed CF anti bodies later but both sera tested contained NT antibodies (titre 40) At follow up 15 of 16 infants positive at admission still were seropositive whereas 1 (case 5) had become CF seronegative Significant changes in CF titre (≥4 fold rise or fall) were seen in only 7 patients All mothers to infants with viruria were themselves seropositive

In the age groups 6–12 months 24 infants were seropositive (Table 2) Virus isolation attempts have only been performed one or two times in 22 of these infants CMV being iso lated from 13 (Table 4) Thus 60% of the sero positive infants in the age group 6–12 months were virus excreters

Table 2 *Results of serologic studies of infants on admission*

Age (months)	<1	1	2–3	4–5	6–12	Total
No positive	32	21	19	5	24	101
No studied	64	50	69	25	102	310
% positive	50	42	28	20	24	33

Table 3 *Serologic studies of mother infant couples with seropositive mothers*[a]

Infants age (*months*)	No posi tive	No stud ied	posi tive	Mean CF log titre ±1 S D: Positive infants	Mothers of positive infants
<1	24	26	92	3 6±1 3	4 3±1 7
1	14	18	78	2 4±1 0	4 8±1 0
2–3	15	28	54	2 8±1 2	4 9±1 6
4–5	3	17	18	2 7±2 1	4 3±0 6
6–12	22	64	34	3 6±1 4	4 6±1 3
Total	78	153	51		

[a] Only 3 infants including 1 pair of twins from 98 sero-negative mothers were seropositive These infants were in the age groups <1 and 1 month when studied sero-logically and the titres were 2 8 and 8

Table 4 *Clinical diagnosis and other data in 21 infants with CMV infection*

Case no	Age (mo) h	Age (mo) f	Clinical diagnosis	Hepatomegaly h	Hepatomegaly f	Resp infect[a] h→f	Follow up	CF titre h	CF titre f	Virus isolation h	Virus isolation f
1	2	5	Acute bronchit febrile convuls				Well	8	64	+	+
2	3	9	Bronchopneumonia laryngeal stridor		+	+	Well apart from slight stridor	32	16	+	+
3	8	15	Acute bronchit slight hydroceph				Well	8	8	–	+
4	8	19	Recurrent bronchit				Well	16	32	+	+
5	8	20	Acute bronchitis		+		Well	8	<2	–	+
6	8	15	Acute resp infect empyema				Well	16	2	+	+
7	8	13	Acute febrile bronchitis	+	+		Well	32	16		+
8	9	12	Acute resp infect febrile convuls				Not examined	16			+
9	10	21	Recurrent bronchit				Well	<2	8	+	–
10	11	20	Recurrent resp inf febrile convulsions		+	+	Well	16	8	–	+
11	11	22	Acute myocarditis resp infect	+	+		Well	8	16	(+)[c]	+
12	6	10	Spastic tetrapleg (acute bronchit at 2 mo)				Severe encephalopathy	64	32	+[d]	+
13	6	12	Encephalopathy motoric retardation	+			Moderate psychomot retard	32		–	+
14	8	21	Motoric retardation		+		Well	64	32	–	+
15	¾	8	Cong heart dis	+	+	+	Conditions unchanged	32	8	+	+
16	9	20	Cong heart dis (recur resp inf)				Not examined	16			+
17	9	11	Cong heart dis (recur resp inf)	+		+	Conditions unchanged	32[b]	16		+
18	2	5	Hiatus hernia	+			Well	8	4	+	+
19	3	6	Cardia insuff			+	Well	4	16	+	+
20	4	—	Wilms tumor cong defect of forearm				Dead 5 mo old	4		+	
21	4	16	Diffuse haemangiomata hemiatrophy			+	Conditions unchanged	32	2	+	+

h On hospitalization *f* At follow up
[a] Repeated respiratory infections in interval between hosp and follow up
[b] Seronegative 6 days old
[c] The strain lost during 1st passage
[d] CMV uria in mother

Clinical findings

The clinical diagnosis and other data concerning the 21 infants with CMV infection characterized by CMV uria are given in Table 4 None of the infants had had classical features of CMV infection in the neonatal period In at least half of the patients (11 of 21), the clinical diagnosis was either acute or recurrent respiratory tract infection (in case 11 associated with acute myocarditis) Most of these patients were in the age group of 6 to 12 months The diagnoses of the other 10 patients included encephalopathy (cases 12–14) congenital heart disease (cases 15–17) and other conditions (cases 18–21) Also a few of these patients had suffered from respiratory tract infection before admission In one patient with severe encephalopathy (case 12) CMV uria was found in the mother

All in all CMV infection was found in 23% of infants admitted because of respiratory tract infections in 9% of infants with neurologic

diseases but in only 5–3° of infants admitted due to congenital heart disease and other diseases (Table 5)

During admission or on follow up hepatomegaly was found in 8 of the 17 infants excluding patients with congenital heart disease Two of the infants (cases 7 and 11) had an enlarged liver for more than 5 and 11 months respectively In the interval between hospitalization and follow up 6 of the 18 infants studied suffered from repeated respiratory tract infections

Follow up examination of the infants who had had respiratory tract infections during admission to hospital revealed that all of them were completely healthy

DISCUSSION

The present investigation of the incidence of CMV infection in infants less than 1 year of age was performed using the most commonly employed methods for diagnosing CMV infection examination of the urine for CMV and the serum for CF antibodies While the first technique only gives the number infected with viruria and thus a minimum estimate of the frequency of infection the serological method employed in infants during the first months of life gives the number of infants who are seropositive after an infection as well as those who have been passively immunized by maternal antibodies

Only 1 of the infants less than 2 months of age excreted CMV in the urine whereas viruria could be demonstrated in almost 6 fold as many infants between 2 and 12 months of age (Table 1) These results are in agreement with those of Starr & Gold (16) and of Stern in hospitalized infants (17) they are also in accordance with the observations of Levinsohn et al (14) on the incidence of CMV infection in non hospitalized infants The increasing frequency of viruria after the age of 2 months seems to provide evidence for the occurrence of early acquired post natal infections Viruria in these infants appears to be relatively prolonged judging from the results of the re isolation studies just as is the case with the viruria of congenital infections (20) and in institutionalized children (9) In addition it should be noted that CMV was isolated less often from the throat than from the urine in the reisolation studies

The serological studies demonstrated good agreement between the serological findings in mothers and newborn infants This together with the decreasing frequency of seropositive infants during the following months and the decrease in the mean titre in these infants strongly suggests that the antibodies in most of the seropositive infants in the youngest age group were maternal The lowest relative number of seropositivity was found in the 4–5 month age group As CMV excretion was demonstrated in 2 of the 5 seropositive infants (40%) in the 4–5 month age group and in 60 of the seropositive infants in the 6–12 months age group it may be surmised that infants with CF antibodies in their serum after the age of 4 months for the most part are seropositive as the results of infection with CMV Maternal antibodies against CMV thus seem to be demonstrable only during the first few months after birth This observation is in agreement with the findings of Carlstrom et al (7) and with the rapid fall in the level of

Table 5 *Distribution of CMV excreters in relation to clinical diagnosis*

Clinical diagnosis	Total[a] studied for CMV uria	CMV uria On admiss	CMV uria At follow up	Total ( )
Respiratory infections	48	5	6	11 (23)
Neurological disease	32	1	2	3 (9)
Congenital heart disease	57	1	2	3 (5)
Other conditions	129	4	0	4 (3)
Total	266	11	10	21

No studied on admission and/or at follow up

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5	8	20	Acute bronchitis		+		Well	8	<2	–	+
6	8	15	Acute resp infect empyema				Well	16	2	+	+
7	8	13	Acute febrile bronchitis	+	+		Well	32	16		+
8	9	12	Acute resp infect febrile convuls				Not examined	16			+
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DISCUSSION

The present investigation of the incidence of CMV infection in infants less than 1 year of age was performed using the most commonly employed methods for diagnosing CMV infection: examination of the urine for CMV and the serum for CF antibodies. While the first technique only gives the number infected with viruria and thus a minimum estimate of the frequency of infection, the serological method employed in infants during the first months of life gives the number of infants who are seropositive after an infection as well as those who have been passively immunized by maternal antibodies.

Only 1 of the infants less than 2 months of age excreted CMV in the urine, whereas viruria could be demonstrated in almost 6 fold as many infants between 2 and 12 months of age (Table 1). These results are in agreement with those of Starr & Gold (16) and of Stern in hospitalized infants (17); they are also in accordance with the observations of Levinsohn et al. (14) on the incidence of CMV infection in non hospitalized infants. The increasing frequency of viruria after the age of 2 months seems to provide evidence for the occurrence of early acquired post natal infections. Viruria in these infants appears to be relatively prolonged judging from the results of the re isolation studies, just as is the case with the viruria of congenital infections (20) and in institutionalized children (9). In addition it should be noted that CMV was isolated less often from the throat than from the urine in the reisolation studies.

The serological studies demonstrated good agreement between the serological findings in mothers and newborn infants. This, together with the decreasing frequency of seropositive infants during the following months and the decrease in the mean titre in these infants, strongly suggests that the antibodies in most of the seropositive infants in the youngest age group were maternal. The lowest relative number of seropositivity was found in the 4–5 month age group. As CMV excretion was demonstrated in 2 of the 5 seropositive infants (40 %) in the 4–5 month age group and in 60 % of the seropositive infants in the 6–12 months age group, it may be surmised that infants with CF antibodies in their serum after the age of 4 months for the most part are seropositive as the results of infection with CMV. Maternal antibodies against CMV thus seem to be demonstrable only during the first few months after birth. This observation is in agreement with the findings of Carlstrom et al. (7) and with the rapid fall in the level of

maternal immunoglobulins which takes place in the course of the first 3–4 months of extra uterine life (13)

One infant (case 9) was CF negative at 10 months of age when CMV could be isolated from the urine and another infant (case 5) with demonstrable viruria at re investigation became seronegative during the period of study Furthermore, the CF titres of several infants were rather low in spite of active infection characterized by CMV uria, and significant changes in CF titre were only seen in a minority These findings indicate, as shown earlier (3, 11) that more than one serologic method for the diagnosis of CMV infections should be employed in infants and children suspected of having CMV disease The two additional methods of choice appear to be the immunofluorescent and the NT test both of which seem to be very specific and sensitive (1, 11) These methods are especially useful together with the CF test for the determination of antibodies in CMV infection as it has been found that rises in the three antibodies seem to be independent and displaced in time The rise in immunofluorescent antibodies has been found to occur prior to rise in CF antibodies (11), which again is known to occur prior to rise in NT antibodies (3)

Taking into account the above-mentioned considerations concerning the rather short persistence of maternal antibodies in infants together with the fact that all but one of the infants with CMV uria also had CF antibodies the frequency of CF seropositive infants above the age of 4 months thus appears to be an expression of the frequency of infection in this group of infants The widespread appearance of maternal antibodies in infants less than 4 months of age makes it impossible to determine the incidence of infection on the basis of serology in this age group and consequently the results of the virus isolation studies must be used

The incidence of CMV infection in the present series of infants appears on basis of the virus isolation studies to be about 1% in infants less than 2 months of age and about 8% in infants aged 2–5 months

Based upon the serologic results the incidence of CMV infection was about 24% in infants between the ages of 6 and 12 months This is in agreement with what has been found elsewhere on the European Continent (5 6 19), but is different from the results of some surveys performed in Japan USA, and Britain (8 10, 18)

CMV infection during the first year of life was in our series about five times more frequent in infants admitted due to infections of the respiratory tract than among infants admitted because of other diseases

The common occurrence of CMV infection among infants with respiratory tract infections may suggest that such infection can be caused by CMV in infancy These respiratory tract infections may be the result of CMV infection alone or in combination with other pathogens (12)

Similar observations have been made by Stern (17) and in adults it is known that CMV can cause pulmonary infections (4)

On the other hand, it is not likely that there is an aetiological relationship between any of the other group of diseases in this series and CMV An exception may be the previously mentioned case of encephalopathy in which CMV uria could be demonstrated in the infant s mother In the remaining cases the CMV infection may be regarded as an accidental finding perhaps an expression of the frequency of silent CMV infections accompanied by viruria in that age group

SUMMARY

Among 357 infants less than 1 year of age a study was undertaken during admission to hospital to determine the incidence of cytomegalovirus (CMV) infection using virological and serological methods

Based on results of virus isolation attempts the incidence of CMV infection was about 1% in infants less than 2 months and 8% in

infants 2–5 months of age whereas the serologic results indicated an incidence of about 24% in the age group 6–12 months

The serological findings suggested that maternal CF antibodies against CMV are demonstrable only during the first few months of life and that the finding of CMV CF sero antibodies in infants above the age of 4 months is the expression of an active infection with this virus As two infants were CF seronegative during a period with active infection characterized by CMV uria it is concluded that the test for CF antibodies is not quite sufficient for serological diagnosis of CMV infection in infancy For that reason other specific serological methods such as the immunofluorescent and virus neutralization tests should be used as a supplement to the CF test

The clinical manifestations in the 21 infants excreting CMV are mentioned Half of the patients were admitted because of respiratory tract infections CMV infection was about 5 fold more frequent in these infants than among infants admitted because of other diseases suggesting that these infections can be caused by CMV in infancy

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One infant (case 9) was CF negative at 10 months of age when CMV could be isolated from the urine and another infant (case 5) with demonstrable viruria at re investigation became seronegative during the period of study Furthermore, the CF titres of several infants were rather low in spite of active infection characterized by CMV uria, and significant changes in CF titre were only seen in a minority These findings indicate as shown earlier (3, 11) that more than one serologic method for the diagnosis of CMV infections should be employed in infants and children suspected of having CMV disease The two additional methods of choice appear to be the immunofluorescent and the NT test both of which seem to be very specific and sensitive (1, 11) These methods are especially useful together with the CF test for the determination of antibodies in CMV infection as it has been found that rises in the three antibodies seem to be independent and displaced in time The rise in immunofluorescent antibodies has been found to occur prior to rise in CF antibodies (11), which again is known to occur prior to rise in NT antibodies (3)

Taking into account the above-mentioned considerations concerning the rather short persistence of maternal antibodies in infants together with the fact that all but one of the infants with CMV uria also had CF antibodies the frequency of CF seropositive infants above the age of 4 months thus appears to be an expression of the frequency of infection in this group of infants The widespread appearance of maternal antibodies in infants less than 4 months of age makes it impossible to determine the incidence of infection on the basis of serology in this age group and consequently the results of the virus isolation studies must be used

The incidence of CMV infection in the present series of infants appears on basis of the virus isolation studies to be about 1% in infants less than 2 months of age and about 8% in infants aged 2–5 months

Based upon the serologic results the incidence of CMV infection was about 24% in infants between the ages of 6 and 12 months This is in agreement with what has been found elsewhere on the European Continent (5 6, 19) but is different from the results of some surveys performed in Japan USA, and Britain (8, 10, 18)

CMV infection during the first year of life was in our series about five times more frequent in infants admitted due to infections of the respiratory tract than among infants admitted because of other diseases

The common occurrence of CMV infection among infants with respiratory tract infections may suggest that such infection can be caused by CMV in infancy These respiratory tract infections may be the result of CMV infection alone or in combination with other pathogens (12)

Similar observations have been made by Stern (17) and in adults it is known that CMV can cause pulmonary infections (4)

On the other hand, it is not likely that there is an aetiological relationship between any of the other group of diseases in this series and CMV An exception may be the previously mentioned case of encephalopathy in which CMV uria could be demonstrated in the infant s mother In the remaining cases the CMV infection may be regarded as an accidental finding, perhaps an expression of the frequency of silent CMV infections accompanied by viruria in that age group

SUMMARY

Among 357 infants less than 1 year of age, a study was undertaken during admission to hospital to determine the incidence of cytomegalovirus (CMV) infection using virological and serological methods

Based on results of virus isolation attempts the incidence of CMV infection was about 1% in infants less than 2 months and 8% in

infants 2–5 months of age whereas the serologic results indicated an incidence of about 24% in the age group 6–12 months

The serological findings suggested that maternal CF antibodies against CMV are demonstrable only during the first few months of life and that the finding of CMV CF sero antibodies in infants above the age of 4 months is the expression of an active infection with this virus As two infants were CF seronegative during a period with active infection characterized by CMV uria it is concluded that the test for CF antibodies is not quite sufficient for serological diagnosis of CMV infection in infancy For that reason other specific serological methods such as the immunofluorescent and virus neutralization tests should be used as a supplement to the CF test

The clinical manifestations in the 21 infants excreting CMV are mentioned Half of the patients were admitted because of respiratory tract infections CMV infection was about 5 fold more frequent in these infants than among infants admitted because of other diseases suggesting that these infections can be caused by CMV in infancy

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PARENTAL CONSANGUINITY AMONG ISRAELI MENTAL RETARDATES

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The purpose of this article is to report a strikingly high rate of parental consanguinity among some of the largest diagnostic groups of Israeli mental retardates and to interpret its significance for the etiology of mental defect Our data collected from a population with a high prevailing rate of consanguinous marriage, show the relationship of consanguinity trends to etiologic diagnosis to an extent that was impossible in the survey so far performed in western countries and they indicate the great potential value of consanguinity analyses in the study of retardation

Previous surveys have shown that the great majority of retardates are of unknown etiology (1 3, 5, 6) that about a quarter of them have retarded siblings and/or parents (1 5 6) and that about 40% of those with retarded siblings have retarded parents (5)

Suggestively high parental consanguinity rates were observed in some of these groups The 1938 Colchester study (6) showed that about 3 2% of all retardates had consanguinous parents, about double the consanguinity rate of the general population Akesson s survey in South Sweden (1) found first cousin parents in 3 of 60 families with undifferentiated retardation Dewey et al (2) found an increased rate of parental consanguinity among severe familial retardates in Wisconsin This latter study included an attempt to analyze severe idiopathic retardation in the 1938 Colchester study by comparing parental consanguinity in that survey with that of the general English population of the same period

These studies hinted that analysis of consanguinity may be a useful approach to studying the etiologies of mental defect However, its application has awaited clarification of precisely which etiologic groups are associated with parental consanguinity and to what degree This clarification was impossible in countries such as England Sweden and the United States because of the very low rates of consanguinous marriage in these countries

MATERIAL AND METHODS

The case material reviewed here consisted of all the retardates (i e I Q under 70 on standardized psychologic testing by our unit) who were fully examined at the Tel Hashomer Assessment Center for the Retarded in the first five years of its existence from 1963 to 1968 All cases were referred to the Center by community social agencies as possible candidates for institutional placement day care centers sheltered workshops and special schools They usually had come to the attention of the community social agency as a result of referral by a schoolteacher or school psychologic services In the case of pre school children the community social worker was called in on the basis of slow development either by public health nurses by neighborhood physicians or by the parents themselves

Full examination was defined as including at least psychologic examination report of community social worker interviews of parents by both social worker and paediatrician charting of the family tree family and developmental history physical examination neurologic examination study of the ocular fundi

Table 1 *Numbers of families by major diagnostic classification and degree of retardation*

Diagnosis	Mild (*I Q 50–69*)	Severe (*I Q <50*)	Mixed	Total
Major chromosomal anomalities	15	79		94
Infections (meningitis encephalitis cong rubella cong toxoplasmosis)	3	21		24
Physical and chemical trauma	1	7		8
Total obviously nonfamilial causes	19 (6%)	107 (18%)		126 (14%)
Specific heredofamilial diseases	7	14	1	22
Unlabelled syndromes with affected close relatives	5	10	2	17
Nonsyndromic sibling retardation	79	95	12	186
Total familial causes	91 (30%)	119 (21%)	15	225 (25%)
Known pathology but uncertain etiology[b]	7 (2%)	20 (3%)		27 (3%)
Idiopathic	185 (61%)	341 (58%)		526 (58%)
Total	302 (100%)	587 (100%)	15	904 (100%)

Specific heredofamilial diseases found include phenylketonuria Laurence–Moon–Biedl syndrome familial dysautonomia juvenile amaurotic idiocy mucopolysaccharidoses homocystinuria tuberous sclerosis Marfan s syndrome and neurofibromatosis

[b] Pathologic conditions found include cretinism hydrocephalus neural tube malformations Cornelia de Lange syndrome congenital hemihypertrophy and an apparently balanced D/G translocation

urine examination for reducing substances and alpha keto acids urine *paper chromatography* of amino acids indoles and phenols and blood phenylalanine level Dermatoglyphics nuclear sex and karyotypes were often examined as indicated

This report is based on 972 examined cases from 904 families Median age was 8 years and practically all were aged under 20 Males outnumbered females by a 3 2 ratio in all diagnostic groups

The following diagnostic classification was used

1 *Retardation of obviously non familial cause*
This group included all cases of trisomy or chromosomal deletion and all those cases of purulent meningitis *encephalitis craniocerebral* trauma or perinatal damage in which the pathologic episode seemed severe enough to cause brain damage and in which no retarded siblings were *known*

2. *Familial retardation*
This group included all cases of disease (such as phenylketonuria) known to be heredofamilial and all cases with a full sibling who showed convincing evidence of mental retardation This evidence was *provided by information that the* child was in a school or institution for the retarded by reports of school psychological services or in cases of doubt by examination at our Center One family with a retarded mother and no retarded sibling was also included in this group

3 *Retardation associated with known pathology but uncertain etiology*
This group included cretinism hydrocephalus the De Lange syndrome *various* neural tube malformations hemihypertrophy and one case of apparently balanced D/G translocation

4 *Idiopathic retardation*
This group included all cases with unknown cause and uncertain pathology of the CNS

Cases with seizures spasticity hyperkinetic behaviour disorders psychotic tendencies and congenital malformations were not classified separately They were rather *equally distributed* among the various etiologic groups with the exception that seizures spasticity and hyperkinesis were somewhat rarer among cases of Down s syndrome

The data presented are based on the number of families with various types of retardation rather than numbers of cases Multiple cases in a family were found only in the group of familial retardates

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RESULTS

Diagnoses

Table 1 shows the classification of the 904 families by degree of retardation and major diagnostic category Obviously nonfamilial causes of retardation accounted for 126 (14%)

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Table 3 *Families with familial retardation by diagnostic category degree of retardation and parental consanguinity*

Dotted lines indicate division of table for χ^2 calculation

Degree of retardation	Diagnostic category	First cousins	Loosely related	Unrelated	Total	
Mild	Heredofamilial diseases	1	0	6	7	$\chi^2 = 1.97$
	Unlabelled syndromes	3	0	2	5	D F = 1
	Nonsyndromic parents normal	5	6	34	45	$p > 0.1$
	Nonsyndromic parent(s) apparently retarded	2	4	28	34	
	Total	11	10	70	91	
Severe	Heredofamilial diseases	10	0	4	14	$\chi^2 = 14.6$
	Unlabelled syndromes	3	1	6	10	D F = 3
	Nonsyndromic parents normal	21	5	28	54	$p < 0.001$
	Nonsyndromic parent(s) apparently retarded	6	1	34	41	
	Total	40	7	72	119	
Mixed	Heredofamilial diseases	1	0	0	1	
	Unlabelled syndromes	2	0	0	2	
	Nonsyndromic parents normal	2	0	7	9	
	Nonsyndromic parent(s) apparently retarded	0	1	2	2	
	Total	5	1	9	15	

1 Heredofamilial disease of known cause (e g phenylketonuria)

2 Retardation as part of an unclassified or unlabelled familial syndrome

3 Retardation without syndromic findings in siblings only

4 Retardation without syndromic findings proven in at least one sibling and suspected in at least one parent

The last group consisted of those families in whom the clinical impression of the examining staff was that at least one of the parents was retarded This impression was based on the parent s apparently inadequate mentation at interview and/or on evidence of his inability to cope alone with minimal tasks of work travel or household organization Since these parents did not undergo psychologic testing their retardation cannot be proven but the possibility of dominant heredity in these families made their separate analysis advisable

Table 3 details the consanguinity trends among the familial retardates It shows that the families with apparently retarded parents and those with mild retardation have no significant increase in parental consanguinity over the approxamately 10 % rate of first cousin marriages found among nonfamilial retardates All other categories of severe familial retardation and of familial retardation of mixed degree (mild and severe in the same family) show greatly increased parental consanguinity

Israel s population consists of a number of ethnic groups with very different rates of inbreeding (4) We have therefore examined our data to see whether the consanguinity trends observed in Tables 2 and 3 exist within each major ethnic group Tables 4 and 5 show that they do As seen in Table 4 all of the larger ethnic groups show a significant increase of parental consanguinity among severe familial cases and all but the Yemenites show a sig

Table 2 *Number of families by major diagnostic category degree of retardation and parental consanguinity*

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Degree of retardation	Diagnostic category	First cousins	Loosely related	Unrelated	Total	
Mild	Obviously nonfamilial	3	1	15	19	$\chi^2 = 2.94$
	Obviously familial	11	10	70	91	DF = 1
	Pathology of uncertain etiology	0	1	6	7	$0.1 > p > 0.05$
	Idiopathic[a]	36	16	133	185	
	Total	50	28	224	302	
Severe	Obviously nonfamilial	9	12	86	107	$\chi^2 = 40.0$
	Obviously familial[a]	40	7	72	119	DF = 6
	Pathology of uncertain etiology	3	1	16	20	$p < 0.001$
	Idiopathic[a]	52	24	265	341	
	Total	104	44	439	587	

[a] Group with significantly high rate of parental consanguinity

of them and they were far more frequent among mild retardates (18%) than among severe (6%) 94 of all these nonfamilial causes, or 75%, were accounted for by chromosomal abnormalities, 87 of them were cases of Down's syndrome Various kinds of infection and trauma were responsible for the remaining 32 retardates in this group Familial causes were found in 225 or 25% of the families examined This was more common among cases of mild retardation (30%) than among severe (21%) 22 of these families (10%) suffered from known specific heredofamilial diseases of which 19 were characterized by autosomal recessive inheritance In another 17 families (8%) the retardation was associated with syndromic findings (e g cataracts deafness) which were constant for each family The remaining 186 families (82%) showed no specific disease and no syndromic findings The familial nature of their retardation was demonstrated only by the presence of a retarded sibling

In 27, or 3%, of the families, retardation was associated with pathologic conditions such as cretinism or hydrocephalus in which heredofamilial causes for the retardation are neither proven or disproven Hydrocephalus accounts for half of this group and cretinism for a quarter

The largest group listed in Table 1 is that of the idiopathic retardates with no clear indication of the etiology or pathology of their retardation This group comprises 526 cases, or 58% of our material

Consanguinity trends

The frequency of parental consanguinity among the various groups is shown in Table 2. One sees that three groups show a significant increase in rate of first cousin marriage when compared to the rest They are

1 Familial retardates severe (34%)
2 Idiopathic retardates mild (19.5%)
3 Idiopathic retardates severe (15.5%)

All other groups show a rate of close parental consanguinity at or near 10%

In view of the increased parental consanguinity among the more severe cases of familial retardation all familial retardates were analyzed in more detail For this purpose, each degree of retardation was classified into the following four groups

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Mixed	Heredofamilial diseases	1	0	0	1	
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	Total	5	1	9	15	

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Table 4 *Parental consanguinity[a] by ethnic groups and diagnostic classification severe idiopathic cases*

Ethnic group	Familial non syndromic I Q <50 Consanguinous[a]	Familial non syndromic I Q <50 Unrelated	Idiopathic I Q <50 Consanguinous[a]	Idiopathic I Q <50 Unrelated	Obviously non familial all degrees Consanguinous[a]	Obviously non familial all degrees Unrelated	Proportion of consanguinous[a] marriages in population at large[b] ( )
Ashkenazi Jews (European)	2	3	6	93	0	36	1 52
Iraqi Jews	9	7	14	31	4	15	22 8
Yemenite Jews	2	5	6	37	2	12	12 2
Moroccan Jews	3	2	5	16	1	6	9 0
Persian Jews	2	1	10	10	0	5	26 0
Total observed	18	18	41	187	7	74	
Total expected (by percentage of Goldschmidt et al)[b]	5 8	30 2	24 1	203 9	8 5	72 5	
χ^2	30 3		13 3		0 30		
p	<0 001		<0 001		N S		

[a] Consanguinous – first cousins and uncle niece pairs lesser degrees of consanguinity are omitted from all calculations
[b] Population data are taken from survey of Goldschmidt et al (4)

nificant increase among the severe idiopathic cases when these cases are compared either to a control group of nonfamilial retardates (of all degrees of severity) or to the prevailing rates of consanguinous marriages in these same ethnic groups as reported in the survey of Goldschmidt et al (4) Table 5 shows that among the mild idiopathic cases the same trend holds for all ethnic groups but the Ashkenazi Jews of European origin

DISCUSSION

Comparing the various categories of mental retardation with the population at large we have found the following

1 Severe nonsyndromic familial retardates with normal parents show a threefold increase in parental consanguinity

2 Idiopathic retardates of all degrees show a 50% to 80% increase in parental consanguinity

3 Familial retardates whose parents appear retarded show no increase in parental consanguinity

4 Mild familial retardates with normal-appearing parents show no increase in parental consanguinity

5 Cases of retardation caused by chromosomal abnormalities meningitis, encephalities trauma and Kernicterus show no increase in parental consanguinity

The first four findings are of interest the last was to be expected The most reasonable explanation of these findings is that autosomal recessive heredity is the predominant cause of low-grade nonsyndromic familial retardation with normal parents and that it is an important cause of idiopathic retardation of all degrees The consanguinity trends observed are not the result of different ethnic representation in each diagnostic category since they are observed within all of the ethnic groups They are unlikely to be the result of independently increased rates of both consanguinous marriage and retardation in lower socioeconomic classes since the parents who appeared retarded belonged to the lowest socio economic strata and yet they showed no increase in the rate of consanguinous marriage

The finding that there is no increase in parental consanguinity among mild familial

Table 5 Parental consanguinity[a] by ethnic group and diagnostic classification mild idiopathic cases

Ethnic group	Idiopathic I Q 50–69 Consanguinous	Unrelated	Obviously non familial all degrees Consanguinous	Unrelated	Proportion of consanguinous marriages in population at large[b] ( )
Ashkenazi (European) Jews	0	31	0	36	1 52
Iraqi Jews	20	27	4	15	22 8
Yemenite Jews	3	12	2	12	12 2
Moroccan Jews	3	9	1	6	9 0
Persian Jews	3	6	0	5	26 0
Total observed	29	85	7	74	
Total expected (by percentage of Goldschmidt et al)[b]	17 1	96 9	8 5	72 5	
χ^2	9 78		0 30		
p	<0 01		N.S		

[a] Consanguinous = first cousins and uncle niece pairs lesser degrees of consanguinity are omitted from all calculations
[b] Population data are taken from survey of Goldschmidt et al (4)

retardates with normal parents is a peculiar one since mild *idiopathic* cases show such an increase and it is unreasonable to suppose that familial retardates have a lower proportion of hereditary autosomal recessive cases than do idiopathic retardates This difference is probably not a real one the numbers in the familial sample are small and the difference between the consanguinity rates of the two groups does not attain statistical significance What these figures do indicate is that autosomal recessive heredity is not the main cause of mild familial retardation

An interesting aspect of our consanguinity data is their support of the clinical suspicion of mental retardation in parents In 41 of our 95 families with nonsyndromic sibling retardation the paediatrician and social worker of the Assessment Center suspected that at least one of the parents was retarded Since parents underwent no psychologic testing and since many of them came from different cultural backgrounds than that of the clinical personnel the reader s first reaction may be to doubt the reliability of such a judgment The data on parental consanguinity indicate that these families indeed had a different heredity than did those whose parents appeared normal Those families in which parental retardation was suspected had no increase whatsoever in parental consanguinity when compared to the general population This finding is in striking contrast to those families in which the parents seem of normal intelligence it indicates the very different genetic nature of these two groups and suggests that the clinical impression of retardation in parents may have some validity Our data are compatible with either a hypothesis of dominant heredity or a hypothesis of recurrent environmental causation in these families

The association that we have observed between parental consanguinity and various etiologic categories of mental retardation is probably a general one It was observed in each of 5 Israeli ethnic groups which differ greatly from each other in their genetic background It has also been observed among similar groups of retardates in England (6) Sweden (1) and the United States (2) It therefore seems likely to us that the *major* role played by autosomal recessive genes in the etiology of severe sibling retardation with normal parents and their significant role in

Table 4 *Parental consanguinity[a] by ethnic groups and diagnostic classification severe idiopathic cases*

Ethnic group	Familial non syndromic IQ <50		Idiopathic IQ <50		Obviously non familial all degrees		Proportion of consanguinous[a] marriages in population at large[b] ( )
	Consanguinous[a]	Unrelated	Consanguinous[a]	Unrelated	Consanguinous[a]	Unrelated	
Ashkenazi Jews (European)	2	3	6	93	0	36	1 52
Iraqi Jews	9	7	14	31	4	15	22 8
Yemenite Jews	2	5	6	37	2	12	12 2
Moroccan Jews	3	2	5	16	1	6	9 0
Persian Jews	2	1	10	10	0	5	26 0
Total observed	18	18	41	187	7	74	
Total expected (by percentage of Goldschmidt et al)[b]	5 8	30 2	24 1	203 9	8 5	72 5	
χ^2	30 3		13 3		0 30		
p	<0 001		<0 001		N S		

[a] Consanguinous – first cousins and uncle niece pairs lesser degrees of consanguinity are omitted from all calculations
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Comparing the various categories of mental retardation with the population at large, we have found the following

1 Severe nonsyndromic familial retardates with normal parents show a threefold increase in parental consanguinity

2 Idiopathic retardates of all degrees show a 50% to 80% increase in parental consanguinity

3 Familial retardates whose parents appear retarded show no increase in parental consanguinity

4 Mild familial retardates with normal appearing parents show no increase in parental consanguinity

5 Cases of retardation caused by chromosomal abnormalities meningitis, encephalities trauma and kernicterus show no increase in parental consanguinity

The first four findings are of interest the last was to be expected The most reasonable explanation of these findings is that autosomal recessive heredity is the predominant cause of low grade nonsyndromic familial retardation with normal parents and that it is an important cause of idiopathic retardation of all degrees The consanguinity trends observed are not the result of different ethnic representation in each diagnostic category since they are observed within all of the ethnic groups They are unlikely to be the result of independently increased rates of both consanguinous marriage and retardation in lower-socioeconomic classes since the parents who appeared retarded belonged to the lowest socio economic strata and yet they showed no increase in the rate of consanguinous marriage

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	Consan guinous[a]	Un related	Consan guinous	Un related	
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Iraqi Jews	20	27	4	15	22 8
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An interesting aspect of our consanguinity data is their support of the clinical suspicion of mental retardation in parents In 41 of our 95 families with nonsyndromic sibling retarda tion the paediatrician and social worker of the Assessment Center suspected that at least one of the parents was retarded Since parents underwent no psychologic testing and since many of them came from different cultural backgrounds than that of the clinical person nel the reader s first reaction may be to doubt the reliability of such a judgment The data on parental consanguinity indicate that these families indeed had a different heredity than did those whose parents appeared normal Those families in which parental retardation was suspected had no increase whatsoever in parental consanguinity when compared to the general population This finding is in striking contrast to those families in which the parents *seem of normal intelligence* it indicates the very different genetic nature of these two groups and suggests that the clinical im pression of retardation in parents may have some validity Our data are compatible with either a hypothesis of dominant heredity or a hypothesis of recurrent environmental causa tion in these families

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the etiology of idiopathic mental retardation of all degrees, are of international importance

SUMMARY

Parental consanguinity was studied among 972 Israeli retardates and was correlated with their diagnostic classification

A strikingly high consanguinity rate was observed among parents who seemed of normal intelligence and who had two or more children with severe retardation This phenomenon was not found among parents who themselves appeared retarded, nor was it found among families with two or more mildly retarded children A moderate increase in parental consanguinity was found among cases of retardation who had unknown etiology and no retarded siblings

The trends described appear both in the pooled material and in individual ethnic groups They seem to indicate that autosomal recessive heredity plays a predominant role in severe familial retardation with normal parents and a significant one in isolated idiopathic retardation of all degrees

By contrast, recessive heredity seems to play no important role in mild familial retardation, and it probably plays no role whatever in families whose parents appear retarded

ACKNOWLEDGEMENTS

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PILOT STUDIES ON THE FLUORIDE METABOLISM IN INFANTS ON DIFFERENT FEEDINGS

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Recent analyses have shown that the fluoride content of human breast milk is much less than previously accepted—below 0 05 mg/l compared with previously given figures of about 0 2 mg/l (1) The fluoride content of milk is thus of the same order as the ionized fluoride of blood plasma and saliva and milk fluoride has not been found to be measurably influenced by the maternal fluoride ingestion (1) Breast fed infants will thus obtain a constant low supply of fluoride while infants fed modern dry milk formulas diluted with tap water will get a fluoride supply which as a rule will be several times that of the breast fed children in Stockholm with about 0 25 mg F/l water the supply will be about 10-fold in Uppsala with 1 2 mg F/l 40-50 fold and areas with 4-5 mg F/l 150-200 fold that of the breast fed infants

In a comparative investigation of permanent teeth mineralized during periods of predominant formula feeding or breast feeding respectively in Uppsala Ericsson & Ribelius (2 3) found that the high fluoride ingestion by the formula fed children had not caused any damage to the teeth—the occurrence and degree of enamel fluorosis in the formula fed children were only insignificantly greater than in the predominantly breast fed children The high fluoride supply to the formula fed children during a period of mineralization of several permanent teeth should according to broad previous experience give an increased frequency and degree of enamel fluorosis as first symptom if any The near absence of this symptom indicates a high tolerance to fluoride during the first year of life

The metabolism of fluoride has however hardly been studied in infants Since the food of the infant is rich in calcium phosphate there is for example the possibility that comparatively large quantities of fluoride may be excreted with the faeces in combination with unabsorbed calcium phosphate It has therefore been deemed important to determine the intake and excretion of fluoride in breast fed and formula fed infants

The present investigation is to be regarded as a pilot study owing to the special difficulties of these investigations in infants

Part 1 Studies in Stockholm

MATERIAL AND METHODS

The subjects were infants of an age where pure formula feeding is most frequent Only healthy infants free from digestive disorders for whom the diet could without inconvenience be changed from water-diluted dry milk to breast milk and vice versa were accepted Since all supply of food as well as collection of urine and faeces had to take place under strict control the tests were done on children in a small infants home Three healthy boys in the age range 11 days to 2 months 8 days participated Before the start of the

the etiology of idiopathic mental retardation of all degrees, are of international importance

SUMMARY

Parental consanguinity was studied among 972 Israeli retardates and was correlated with their diagnostic classification

A strikingly high consanguinity rate was observed among parents who seemed of normal intelligence and who had two or more children with severe retardation This phenomenon was not found among parents who themselves appeared retarded, nor was it found among families with two or more mildly retarded children A moderate increase in parental consanguinity was found among cases of retardation who had unknown etiology and no retarded siblings

The trends described appear both in the pooled material and in individual ethnic groups They seem to indicate that autosomal recessive heredity plays a predominant role in severe familial retardation with normal parents and a significant one in isolated idiopathic retardation of all degrees

By contrast recessive heredity seems to play no important role in mild familial retardation, and it probably plays no role whatever in families whose parents appear retarded

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were also reduced Breast milk contains considerably less of all these substances than dry milk formulas

The urinary fluoride concentrations during the formula feeding period are considerably less than the concentrations of the formula preparations as may be expected in individuals with rapidly growing skeletons On the other hand urinary fluoride from the breast milk periods is much higher than that of the breast milk and fairly constant during the whole week of experiment 2 This indicates a probably negative fluoride balance which was not levelled out during this week It is well known that adults who change over from a period of high fluoride ingestion to a period of considerably lower supply may be in negative fluoride balance for a long time through relatively high fluoride excretion with the urine The mobilisation of fluoride from the skeleton may be expected to be more pronounced in infants with their rapid remodelling of the skeleton

Table 2 *Concentrations of F in urine after ingestion of breast milk*

Subject/Age/ Day	F ppm	Average
L K 2 mo 24 d		
1	0 08	
2	0 05	
3	0 06	
4	0 08	
5	0 0	0 07
6	0 08	
7	0 07	
J H 2 mo 1 d		
1	0 07	
2	0 07	
3	0 08	
4	0 08	
5	0 06	0 07
6	0 06	
7	0 09	
M O 27 d		
1	0 09	
2	0 05	
3	0 06	
4	0 06	
5	0 06	0 06
6	0 05	
7	0 06	

Table 3 *Concentrations of F Ca and P in faeces and of F in urine after ingestion of water diluted formula*

	Subject/Age Day	Formula (F ppm)	Faeces F ppm	Faeces Ca mg/g	Faeces P mg/g	Urine (F ppm)
Set I	*C E 2 mo 17 d*					
	4	0 99	—	—	—	0 45
	5	0 99	1 94	8 78	0 94	0 40
	6	0 99	—	—	—	0 38
	L K 1 mo 10 d					
	4	0 99	—	—	—	0 32
	5	0 99	1 56	10 38	1 19	0 33
	6	0 99	—	—	—	0 30
Set II	*A M N 2 mo 18 d*					
	4	0 71	—	—	—	0 48
	5	0 71	—	—	—	0 57
	6	0 71	0 83	10 40	1 41	0 49
	U H 1 mo 24 d					
	4	0 71	—	—	—	0 57
	5	0 71	—	—	—	0 55
	6	0 71	0 64	12 11	0 57	0 53

Part 2 Studies in Uppsala

In Uppsala where as pointed out in the introduction the fluoride intake by formula fed children could be expect d to be 40–50 times that of breast fed infants metabolic balance studies were arranged with some infants The e studies were carried out in the metabolic ward of the Children s Clinic of the Academic Hospital except the chemical analyses which were carried out at th Laboratory of Oral Biochemistry in Stockholm

MATERIAL AND METHODS

The ubjects were 4 girls aged between 1 month 10 days and 2 months 18 days at the start of the tests The children had no known disea es or disorders The tests w re done in two sets with two children each

The tests comprised 2 periods (*a*) 6 days on formula feedin, and (*b*) 6 subsequent days on breast milk feeding The metabolic study comprised only the 3 last days of each period in order to let faecal excretion approach balance with the supply of the period

(*a*) *Formula feeding* All the children obtained the same formula (Findus Milkotal) which for every meal (5 daily) was diluted according to th manufacturer s recommendation (by weight 1 part of powder + 6 parts of water) The water mixed formula was fed from a nursing bottle which was weighed before and after the meal

The fluoride content of the tap water as well as of the mixed formula was determined repeatedly during the experiment using the Orion fluoride electrode with ashing of the mixed formula The original fluo-

Table 1 *a* *Concentrations of F, Ca, Mg and P in faeces and of F in urine after ingestion of breast milk*

Subject/ Age/ Day	Breast milk (F ppm)	Faeces F ppm	Faeces Ca mg/g	Faeces Mg mg/g	Faeces P mg/g	Urine (F ppm)
L K 2 mo 8 d						
1	<0 05	0 96	10 50	1 27	0 88	—
2	<0 05	0 60	4 41	0 88	0 67	—
3	<0 05	—	—	—	—	0 08
J H 1 mo 16 d						
1	<0 05	2 80	11 60	1 46	1 91	—
2	<0 05	0 81	6 33	0 97	0 64	—
3	<0 05	1 05	3 85	1 05	0 75	0 05
M O 11 d						
1	<0 05	1 07	27 01	1 76	3 77	—
2	<0 05	0 69	23 09	1 91	3 86	—
3	<0 05	—	—	—	—	0 06

tests the infants were fed water diluted formulas (Similac Milkotal Babysemp) with a fluoride content varying between 0 2 and 0 4 ppm The powders were diluted according to the instructions of the manufacturers (1 + 6 parts by weight) with Stockholm tapwater containing about 0 25 ppm F In consultation with the physician of the home and with the consent of the parents the formulas were replaced for part of the experimental period by breast milk from the Maternal Milk Centre in Stockholm The ingested quantities and the frequencies of ingestion were kept unchanged

The tests were done over two periods the first comprising 6 days (experiments 1 *a* and 1 *b*) the second 7 days (experiment 2)

Table 1 *b* *Concentrations of F Ca Mg and P in faeces and of F in urine after ingestion of water diluted formula*

Subject/ Age/Day	Formula (F ppm)	Faeces F ppm	Faeces Ca mg/g	Faeces Mg mg/g	Faeces P mg/g	Urine (F ppm)
L K 2 mo 8 d						
1	*c* 0 30	0 65	7 40	1 66	1 07	—
2	*c* 0 30	1 45	15 08	2 31	1 67	—
3	*c* 0 30	—	—	—	—	0 19
J H 1 mo 16 d						
1	*c* 0 32	3 24	3 24	0 68	0 81	—
2	*c* 0 32	1 07	15 32	1 72	3 99	—
3	*c* 0 32	0 91	17 33	2 21	4 50	0 13
M O 11 d						
1	*c* 0 28	0 12	10 09	1 19	0 97	—
2	*c* 0 28	—	—	—	—	—
3	*c* 0 28	—	—	—	—	—

In experiment 1 the fluoride excretion in urine and faeces and the excretion of calcium magnesium and phosphate in faeces were studied 1 *a* after change over from formulas to breast milk for 3 days and 1 *b* after immediate return to formula feeding for 3 days In both tests urine was collected for fluoride analysis in special plastic containers which were applied between 10 and 2 o clock on the third day of the respective experiment Samples of faeces were taken at 2 o clock on those days when defecation occurred

In experiment 2 breast milk was fed to the children for a period of 7 days with urine collection every day between 10 and 2 o clock

Urinary fluoride was determined with the Orion fluoride electrode after buffer addition (5)

Faecal samples were homogenized with a Polytron homogenizer after which weighed aliquots were ashed 3 h at 550 C in an oven with minimized risk of contamination F was analysed with the fluoride electrode as above Ca and Mg by atomic absorption according to Willis (9) and inorganic P according to a modification of Kjerulf Jensen s colorimetric method (7) Analyses of fluoride in breast milk from the Maternal Milk Centre in Stockholm and in the formulas used in the tests and the Stockholm tap water had earlier been done repeatedly in our laboratory

RESULTS

The concentrations of F Ca, Mg and P in faeces showed generally decreasing values during the breast milk period (Table 1 *a*) During the formula period on the other hand an increase of Ca Mg and P in faeces was found (Table 1 *b*) The age of the subjects had no apparent influence on these concentrations

In the urine F values more than twice as high were found after 3 days on dry milk formulas as after 3 days on breast milk (Table 1 *a* and 1 *b*) The urinary F excretion during the subsequent 7 day test period with breast milk showed rather constant values during the whole period (Table 2)

DISCUSSION

The decreased faecal excretion of F Ca, Mg and P after change over from formula feeding to breast milk feeding was expected since the concentration of fluoride in the diet was reduced from about 0 30 ppm to about 0 025 ppm and the concentrations of Ca Mg and P

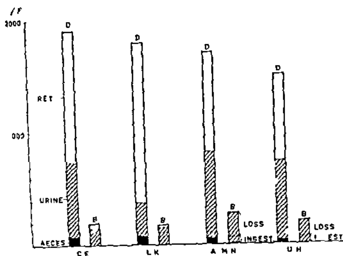


Fig 1 Fluoride metabolism in infants receiving breast milk (B) or dry milk formula (D) diluted with water containing 1 ppm F Total ingestion minus excretion with urine and faeces = retention This was negative i e a loss of fluoride in subjects A M N and U H when on breast milk

while in the second experiment the fluoride retention after breast milk was even negative

The fluoride excretion with the urine was as expected much higher than with the faeces As a rule the ratio urinary F faecal F was much higher than that found in adults with stable fluoride supply

No diurnal variation could be observed as regards the F content of the urine Largely the same F content was found in all the urinary samples from a 24 hour period with formula as well as breast milk diet

The concentration of calcium as well as fluoride in faeces decreased during the breast milk period probably mainly because of the reduced intake of both sub tances

Both ingestion and excretion of fluoride were greater in the older children but otherwise no clear metabolic age differences could be observed

DISCUSSION

There were both expected and unexpected findings in this pilot study It was not surprising that the change from an F rich diet (formula) to F poor breast milk could induce a negative fluoride balance for a certain period of time even if it has also been observed previously that fluoride liberated during the remodelling of a growing bone may be partly retained in newly mineralized parts of the same bone (8)

On the other hand it was hardly expected that the difference in fluoride retention would be so great with varying fluoride supply The similarity of enamel fluorosis index between breast fed and bottle fed children referred to in the introduction is thus not explained by any proportionally increased excretion in the bottle fed children Other hypotheses may be offered as an explanation of the slight effect on enamel mineralization from the greatly increased fluoride dose with formula feeding the rapid skeletal mineralization during the first months of life may particularly rapidly sequester the fluoride from the blood the period of increased fluoride supply may be too short to involve any stronger influence on the ameloblasts a cellular adaptation to fluoride known from experiments with cultivation of bacteria and mammal cells may be particularly effective during this period of life the preferential localization of fluoride induced enamel spots to certain tooth surfaces indicates local factors which may be connected with resorption processes in the roots of deciduous teeth and the jaw bone

Analyses of the F content of enamel and dentine mineralized during periods of breast feeding and formula feeding respectively in Uppsala children have shown an average dif-

Table 4 *Concentrations of F Ca and P in faeces and of F in urine after ingestion of breast milk*

	Subject/ Age/ Day	Breast milk (F ppm)	Faeces F ppm	Faeces Ca mg/g	Faeces P mg/g	Urine (F ppm)
Set I	*C E 2 mo 17 d*					
	4	0 085	0 19	3 29	1 42	0 13
	5	0 085	—	—	—	0 13
	6	0 085	—	—	—	0 13
	L K 1 mo 10 d					
	4	0 085	—	—	—	0 14
	5	0 085	0 13	3 88	0 81	0 14
	6	0 085	—	—	—	0 12
Set II	*A M N 2 mo 18 d*					
	4	0 025	—	—	—	0 13
	5	0 025	—	—	—	0 15
	6	0 025	0 22	7 42	1 20	0 15
	U H 1 mo 24 d					
	4	0 025	—	—	—	0 12
	5	0 025	—	—	—	0 13
	6	0 025	0 25	4 88	0 76	0 13

ride content of the Uppsala water was found to be reduced to a somewhat varying extent by the softening filter of the hospital Twenty four hour urinary volumes were collected in special plastic containers that were applied at every nursing (5 daily) and removed at the subsequent nursing The contents were transferred to plastic bottles and frozen before transport to the laboratory Fluoride was determined on every sample separately with the electrode after buffer addition

Total quantities of faeces including contaminated parts of the cellulose napkins were collected weighed and frozen before transportation to the laboratory where they were ashed and analysed for F Ca and P using the same methods as given above

(*b*) *Breast milk diet* Immediately following the 6-day formula dietary the children were transferred to a 6 day breast milk dietary The milk was delivered from mothers living in Uppsala During the first experimental period the milk was collected and stored in bottles that had been rinsed with Uppsala tap water while for the second period bottles washed and rinsed with deionised water were used The breast milk was given by bottle in the same way as the diluted formula

Fluoride in the breast milk was determined in the same way as in the diluted dry milk formula

RESULTS

From Tables 3–5 and Fig 1 it appears that the differences in fluoride retention between breast milk and formula dietaries is still greater than the difference in supply between the two dietaries The children obtained between 10 and 26 times as much fluoride with the formula as with the breast milk that the difference was not greater was due to the fact that the tap water of the hospital had somewhat lower fluoride content (about 1 ppm) than had previously been analysed in the Uppsala water and that the breast milk of the first experiment had an increased fluoride content obviously from a slight contamination of the storage bottles rinsed in tap water The difference in total retained fluoride between formula and breast milk was considerably higher 28–65 times in the first experiment

Table 5 *Fluoride metabolism after supply of water diluted formula and breast milk respectively 72 h collection time %=per cent of intake*

	Supply	Ingestion (μg)	Urine μg	Urine %	Faeces μg	Faeces %	Retention μg	Retention %
Set I	*C E 2 mo 17 d*							
	Formula	1 903 3	665 7	35 0	70 5	3 7	1 167 1	61 3
	Breast milk	193 8	150 2	77 5	1 2	0 6	42 4	21 9
	L K 1 mo 10 d							
	Formula	1 775 8	295 8	16 7	71 4	4 0	1 408 6	79 3
	Breastmilk	178 6	153 8	86 1	3 2	1 8	21 6	12 1
Set II	*A M N 2 mo 18 d*							
	Formula	1 683 6	755 6	44 9	55 1	3 3	872 9	51 8
	Breastmilk	63 3	262 0		6 8	10 7	−205 5	
	U H 1 mo 24 d							
	Formula	1 482 5	694 9	46 9	31 2	2 1	756 4	51 0
	Breast milk	56 1	191 2		4 5	8 0	−139 6	

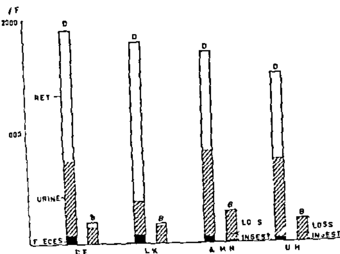


Fig 1 Fluoride metabolism in infants receiving breast milk (B) or dry milk formula (D) diluted with water containing 1 ppm F Total ingestion minus excretion with urine and faeces = retention This was negative i e a loss of fluoride in subjects A M N and U H when on breast milk

while in the second experiment the fluoride retention after breast milk was even negative

The fluoride excretion with the urine was as expected much higher than with the faeces As a rule the ratio urinary F faecal F was much higher than that found in adults with stable fluoride supply

No diurnal variation could be observed as regards the F content of the urine Largely the same F content was found in all the urinary samples from a 24-hour period with formula as well as breast milk diet

The concentration of calcium as well as fluoride in faeces decreased during the breast milk period probably mainly because of the reduced intake of both substances

Both ingestion and excretion of fluoride were greater in the older children but otherwise no clear metabolic age differences could be observed

DISCUSSION

There were both expected and unexpected findings in this pilot study It was not surprising that the change from an F rich diet (formula) to F poor breast milk could induce a negative fluoride balance for a certain period of time even if it has also been observed previously that fluoride liberated during the remodelling of a growing bone may be partly retained in newly mineralized parts of the same bone (8)

On the other hand it was hardly expected that the difference in fluoride retention would be so great with varying fluoride supply The similarity of enamel fluorosis index between breast fed and bottle fed children referred to in the introduction is thus not explained by any proportionally increased excretion in the bottle fed children Other hypotheses may be offered as an explanation of the slight effect on enamel mineralization from the greatly increased fluoride dose with formula feeding the rapid skeletal mineralization during the first months of life may particularly rapidly sequester the fluoride from the blood the period of increased fluoride supply may be too short to involve any stronger influence on the ameloblasts a cellular adaptation to fluoride known from experiments with cultivation of bacteria and mammal cells may be particularly effective during this period of life the preferential localization of fluoride induced enamel spots to certain tooth surfaces indicates local factors which may be connected with resorption processes in the roots of deciduous teeth and the jaw bone

Analyses of the F content of enamel and dentine mineralized during periods of breast feeding and formula feeding respectively in Uppsala children have shown an average dif

Table 4 *Concentrations of F Ca and P in faeces and of F in urine after ingestion of breast milk*

Subject/ Age/ Day	Breast milk (F ppm)	Faeces F ppm	Faeces Ca mg/g	Faeces P mg/g	Urine (F ppm)
Set I *C E 2 mo 17 d*					
4	0 085	0 19	3 29	1 42	0 13
5	0 085	—	—	—	0 13
6	0 085	—	—	—	0 13
L K 1 mo 10 d					
4	0 085	—	—	—	0 14
5	0 085	0 13	3 88	0 81	0 14
6	0 085	—	—	—	0 12
Set II *A M N 2 mo 18 d*					
4	0 025	—	—	—	0 13
5	0 025	—	—	—	0 15
6	0 025	0 22	7 42	1 20	0 15
U H 1 mo 24 d					
4	0 025	—	—	—	0 12
5	0 025	—	—	—	0 13
6	0 025	0 25	4 88	0 76	0 13

ride content of the Uppsala water was found to be reduced to a somewhat varying extent by the often ing filter of the hospital Twenty four hour urinary volumes were collected in special plastic containers that were applied at every nursing (5 daily) and re moved at the subsequent nursing The contents were transferred to plastic bottles and frozen before trans port to the laboratory Fluoride was determined on every sample separately with the electrode after buffer addition

Total quantities of faeces including contaminated parts of the cellulose napkins were collected weighed and frozen before transportation to the laboratory where they were ashed and analysed for F Ca and P using the same methods as given above

(*b*) *Breast milk diet* Immediately following the 6-day formula dietary the children were transferred to a 6 day breast milk dietary The milk was delivered from mothers living in Uppsala During the first experi mental period the milk was collected and stored in bottles that had been rinsed with Uppsala tap water while for the second period bottles washed and rinsed with deionised water were used The breast milk was given by bottle in the same way as the diluted for mula

Fluoride in the breast milk was determined in the same way as in the diluted dry milk formula

RESULTS

From Tables 3–5 and Fig 1 it appears that the differences in fluoride retention between breast-milk and formula dietaries is still greater than the difference in supply between the two dietaries The children obtained be tween 10 and 26 times as much fluoride with the formula as with the breast milk that the difference was not greater was due to the fact that the tap water of the hospital had some what lower fluoride content (about 1 ppm) than had previously been analysed in the Upp-sala water and that the breast milk of the first experiment had an increased fluoride con tent, obviously from a slight contamination of the storage bottles rinsed in tap water The difference in total retained fluoride between formula and breast milk was considerably higher 28–65 times in the first experiment,

Table 5 *Fluoride metabolism after supply of water diluted formula and breast milk respectively 72 h collection time %=per cent of intake*

	Supply	Ingestion (µg)	Urine µg	Urine %	Faeces µg	Faeces %	Retention µg	Retention %
Set I	*C E 2 mo 17 d*							
	Formula	1 903 3	665 7	35 0	70 5	3 7	1 167 1	61 3
	Breast milk	193 8	150 2	77 5	1 2	0 6	42 4	21 9
	L K 1 mo 10 d							
	Formula	1 775 8	295 8	16 7	71 4	4 0	1 408 6	79 3
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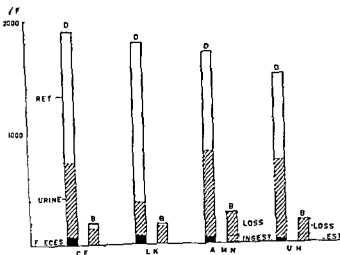


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CASE REPORT

KETOTIC HYPOGLYCEMIA IN A FOUR YEAR OLD BOY WITH ADRENAL CORTICAL INSUFFICIENCY

ODDMUND SØVIK SVEIN OSEID and JOSTEIN VIDNES

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Ulstrom and associates have described a syndrome of sporadic and symptomatic hypoglycemia associated with ketosis in children (3) Episodes of hypoglycemia could be provoked by feeding a low-calorie diet and were characterized by a failure to respond to glucagon This type of hypoglycemia usually occurred after infancy and in children with a history of low birth weight Approximately half of the children with idiopathic hypoglycemia were of the ketotic type Grunt et al (9) studying eight children with ketotic hypoglycemia added the following characteristics a stormy perinatal course a marked variability in blood sugar levels during a 12 hour fast and rapid utilization of glucose despite normal to low levels of serum insulin The pathogenesis of this disorder is however still obscure and the existence of this disease entity is questioned by the work of Senior & Loridan (16) In the latter study the affected children as well as the control subjects developed hypoglycemia in response to a ketogenic diet In pituitary insufficiency the association of hypoglycemia and ketonuria has been observed (15 17)

We here report on a case of ketotic hypoglycemia in which the diagnostic work up revealed a primary adrenal cortical insufficiency (Addison's Disease)

METHODS

Heparinized venous blood was collected on ice and the plasma was separated by centrifugation as soon as possible The plasma was kept at −20 C until analyzed Blood sugar was determined by the method of Hultman (12) after deproteinization with trichlor acetic acid Free fatty acids (FFA) were assayed as described by Dole (5) as modified by Trout (18) ketone bodies according to Pawan (14) Lactic acid was determined by the UV method of Hohorst et al (11) Serum electrolytes were determined at the Central Laboratory Rikshospitalet 17 OH corticosteroids (17 OHCS) in urine were determined at the Division of Endocrinology Medical Department B Rikshospitalet (2 6) aldosterone at the Hormone Laboratory Haukeland Hospital Bergen Immunoreactive insulin (IRI) was determined by the method of Hales & Randle (10) as described in detail in Technical bulletin 68/6 the Radiochemical Centre Amersham

CASE REPORT AND RESULTS

Boy 4 $/_{12}$ years He was full term with a weight of 3 700 g Attacks of vomiting started at the end of his fourth year of life During the attacks he became irritable with weakness and loss of appetite A beneficial effect of extra salt intake was noticed He was first admitted to our hospital aged 4 $/_{12}$ years due to vomiting Sodium and chloride in serum were low there was hypoglycemia (38 mg/100 ml) ketonuria and slight metabolic acidosis He improved rapidly after glucose and saline i v Later he was readmitted several times with similar symptoms and once he had convulsions As shown in Fig 1 fasting was tolerated well for about 12 hours Thereupon a rapidly decreasing blood sugar was observed As shown in Table 1 prolonged fasting caused a rather marked ketonemia, and also some increase of serum

ference of only the order of 2–3, thus much less than the differences in F retention (Ericsson, in press)

It is finally of interest to calculate the maximal F concentration of bone mineral crystallized during formula feeding of Uppsala children The maximal daily F retention of about 470 μg F (subject L K), if entirely taken up by 1 25 g bone mineral crystallized daily (6) would correspond to less than 0 04% F (400 ppm) The safe upper F limit in infant bone is not known, but in adults the first symptoms of osteosclerosis are normally found at about 0 5% F in the bones

SUMMARY

Studies on fluoride (F) excretion and retention have been carried out on seven infants aged between 11 days and 2 months 18 days

In an infants home spot samples of urine and faeces were taken from three infants who were for subsequent periods of varying length on either a formula diet or breast milk The F concentrations of these diets were about 0 3 and below 0 05 ppm respectively Urinary F concentrations were lower than those ingested with the diluted formulas but higher than those ingested with the breast milk indicating a retention and loss of F, respectively during these different feeding periods The negative F balance persisted for at least a week after change over from formula to breast milk feeding

In a metabolic ward the F quantities ingested and those excreted with urine and faeces were determined in four infants who were first on their ordinary formula diet, then on breast-milk diet for 6 days The sampling periods comprised the last 3 days of each feeding period The softened city water used for dilution of the dry milk formula contained about 1 ppm F, and with this feeding two of the infants ingested around 10 times and the other two about 25 times as much F per day as during the breast milk period The difference in F retention was found to be still greater, there being actually a negative F balance in the two infants on the lowest F ingestion with breast milk

Faecal F excretion was consistently low, and both F, Ca, Mg and P were lower in faeces from the breast milk periods than from the formula periods

The desirability of more detailed studies of F metabolism in infants is pointed out

ACKNOWLEDGEMENTS

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Key words Fluoride infants ingestion excretion retention

this was confirmed by direct measurement of ACTH in plasma The plasma level was extremely high and compatible with three conditions only e g primary adrenal cortical insufficiency Cushings disease (post adrenalectomy) and ectopic ACTH secreting tumor Since the second possibility could be excluded and there were no evidence of an ACTH producing tumor we are left with an adrenal cortical insufficiency of unknown origin

Addison s disease appears to be a rare disorder in childhood In reviewing the literature up to 1966 D Albora & Martin (4) found only 106 well-documented cases in children under 15 years of age Among these cases hypoglycemic convulsions were rare as presenting symptoms However of the 2 cases presented by D Albora & Martin one case was characterized by hypoglycemia hyperpigmentation and loss of prompt water diuresis but without renal electrolyte losses and dehydration, suggesting a loss of cortisol production mainly The other case showed signs of aldosterone deficiency mainly with vomiting and dehydration hyperkalemia and hypochloremic acidosis Migeon and coworkers (13) found hypoglycemia as the initial symptom in 3 of 5

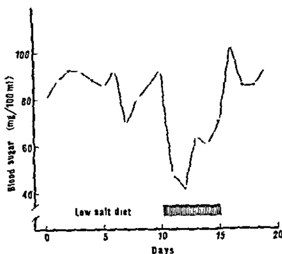


Fig 2 Fasting blood sugar on standard and low salt diet The low salt diet was made by preparing fresh food without adding salt Capillary blood was obtained after fasting overnight and analyzed for sugar as mentioned in text The low salt diet was given in the time period indicated by the hatched area

children with isolated cortisol deficiency These authors found normal electrolyte metabolism in their patients and on the basis of their findings they postulated a congenital unresponsiveness to ACTH

The ketotic type of hypoglycemia in the present case was most probably caused by a defective production of glucocorticoids in the adrenals There was no hyperinsulinism during the development of hypoglycemia and there was a norm l response to glucagon after a 12 hour fast indicating a normal ability to mobilize liver glycogen

The decreasing blood sugar during salt restriction is interesting It is possible that loss of appetite and reduced food intake on a low salt diet precipitated hypoglycemia Alternatively there might be some hitherto unknown biochemical linkage between glucose homeostasis and salt metabolism Clearly salt deprivation was triggering his hypoglycemia

From experimental work it is well known that glucocorticoids cause a rapid increase of glucose production in the liver by inhibiting glycolysis and by providing gluconeogenic precursors Furthermore the steroid hormones

Table 3 *Electrolytes in serum and urine and aldosterone in urine on standard and low salt diet*

The low salt diet was made by preparing fresh food without adding salt Samples of urine and serum were obtained in the morning and analyzed for electrolytes as mentioned in text Aldosterone was determined in 24 hours urine samples

	Serum mEq/l		Urine mEq/l			
Days	Na +	Cl	Na +	Cl −	K +	Aldosterone (µg/24 hours)
Standa d salt						
1	140	107				
2			165	72	42	0 8
3	141	105				
6	141	109				
Low salt						
8	134	104				
9	136	100	176	146	38	
10	136	94	138	116	64	
11	1 8	92	74	20	24	
12			21	13	15	
13						1 3

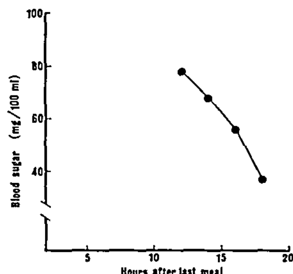


Fig 1 Development of hypoglycemia upon fasting The patient fasted after at least 3 days on a diet with sufficient carbohydrates During the test he was in bed and only allowed water Blood was obtained at the time periods indicated and analyzed for sugar as mentioned in text

FFA Plasma IRI varied somewhat with one slightly elevated value (24 μU/ml) A glucagon tolerance test after fasting overnight showed a normal response with a blood sugar increase from 80 mg per 100 ml in the fasting state to 136 mg per 100 ml 20 min after i v injection of glucagon (0 02 mg/kg) During re admission a blood pressure of 95/60 and hyperpig mentation of the skin was noticed and the patient was therefore studied further from the point of view of adrenocortical insufficiency There were rather low levels of 17 OHCS in the urine (Table 2) After 1 mg Synacthen® (Tetracosactidi hexaacetas) daily for 3 days the urinary output of 17 OHCS was unchanged The ability to conserve electrolytes on a low salt diet was studied As shown in Table 3 sodium and chloride in serum as well as urine decreased The urinary output of aldosterone tended to be increased On a low salt diet there was a definite tendency to-

Table 1 *Plasma insulin ketone bodies and FFA on prolonged fasting*

Venous blood was obtained at the time periods indicated The analytical procedures were carried out in duplicate as described in text The corresponding blood sugar values are presented in Fig 1

	Hours after last meal			
	12	14	16	18
Plasma insulin μU/ml	4	24	3	15
Ketone bodies mg/ml	0 8	2 7	5 0	9 8
FFA mEq/l	0 8	1 0	—	1 4
Lactic acid mg/100 ml	—	4 0	—	4 8

Table 2 *17 OHCS in urine and plasma before and after injection of synthetic ACTH*

0 5 mg Synacthen was injected i m twice daily for 2–3 days Samples of 24 hours urine were assayed for 17 OHCS as mentioned in text The test was repeated 2 months after treatment with Synacthen twice weekly (lower part of the table)

Days	Synthetic ACTH	17 OHCS in urine (mg/24 hours)
1	0	0 1
2	+	0 1
3	+	0 3
4	+	0 1
5	0	0 1
1	0	0 1
2	0	0 1
3	+	—
4	+	0 3
5	0	0 3

ward hypoglycemia with rapid improvement after returning to standard diet (Fig 2) Treatment was started with cortisone and additional salt and he was discharged Since there was a possibility that he suf fered from a pituitary lesion he received injections of Synacthen® twice a week On readmission 2 months later he had been without symptoms Cortisone was temporarily discontinued and he was again tested with Synacthen for 2 days As shown in Table 2 the 17 OHCS of urine were not significantly changed during the test

Plasma obtained before the treatment started was assayed for ACTH activity through the kindness of Dr J G Ratcliffe Department of Chemical Pathology St Bartholomews Hospital London While normal adult morning levels are generally no higher than 80 pg per ml this case showed a level of 1 306 pg per ml

DISCUSSION

The case presented showed several symptoms and signs of adrenal cortical insufficiency (Addison s disease) There were periods of vomiting increased intake of salt hypogly cemia hypotension and hyperpigmentation Apparently, there was some loss of glucocorti coid as well as mineralcorticoid activity Since there was poor response of urinary 17 OHCS to injections of synthetic ACTH, even after prolonged pretreatment with this drug it was concluded that the insufficiency was not secondary to loss of pituitary ACTH Later

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raise the activity of the gluconeogenic enzymes, probably through a stimulation of the enzyme forming systems (1) Adrenalectomy, on the other hand, leads to a decreased output of glucose from the rat liver (8) Experimental work also indicates that the stimulatory effect of glucagon on gluconeogenesis requires steroids Thus the stimulating effect of glucagon on glucose production in the perfused rat liver is almost abolished after adrenalectomy, and is restored by giving dexamethasone to the animals more than 2 hours prior to the test (7) A similar permissive effect of the steroids on glucagon or epinephrine-induced glyco genolysis in rat liver has also been demonstrated (7) Glucocorticoids might also in fluence glucose homeostasis by controlling the synthesis of epinephrine in the adrenal medulla (19)

The present work clearly indicates that lack of adrenal steroids may lead to ketotic hypoglycemia In general, however, no impairment of adrenal cortical function has been detected in this disorder (3) Cortisone treatment, on the other hand, is of importance in preventing symptoms Thus in the original work of Colle & Ulstrom (3) all of the children who had reacted to the provocative diet with symptomatic hypoglycemia were completely protected when cortisone pretreatment was given This effect of cortisone would suggest a defect in gluconeogenesis a possibility examined by Senior & Loridan (16) In the study of glucose production from glycerol no impairment of gluconeogenesis in children with ketotic hypo glycemia could be detected Since glucose production from amino acids was not studied the work by Senior & Loridan is not conclusive with respect to the role of gluconeogenesis in ketotic hypoglycemia

SUMMARY

A boy aged 4 years and 3 months presented symptoms including hypoglycemia ketosis and convulsions On prolonged fasting his blood sugar fell below 40 mg per 100 ml, he developed marked ketonemia, and showed a slight increase in serum FFA Hypotension and hyperpigmentation pointed to an adrenal corti cal insufficiency A poor response of urinary 17 OHCS to ACTH supported this diagnosis Determination of plasma ACTH showed a markedly elevated value, excluding a pituitary defect It is concluded that the ketotic variety of hypoglycemia in the present case was caused by adrenal cortical insufficiency with lack of glucocorticoids and reduced gluconeogenesis The findings are discussed in view of recent studies on the role of glucocorticoids in the control of hepatic gluconeogenesis The important role of the adrenal steroids in main taining normoglycemia is stressed

ACKNOWLEDGEMENT

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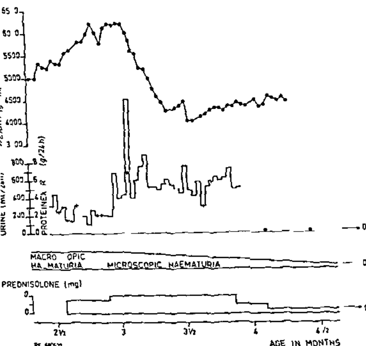


Fig 1 Steroid treatment and course Urine findings essentially normal at about 6 months

and most interesting fact is that Huldt prompted by the present case induced infection with a low virulent toxoplasma strain in mice and could then demonstrate antigen antibody complexes in the glomerulus (3) Later she has found in rabbits that complement is part of such complexes (4) Thus there is some clinical and experimental support for considering the possibility of a connection between the two diseases of our patient The nature of the renal disease and the apparently good prognosis would be compatible with an immune complex nephritis similar to post streptococcal nephritis but with *Toxoplasma gondii* as the relevant antigen The pathogenesis of the anaemia and the thrombocytopenia

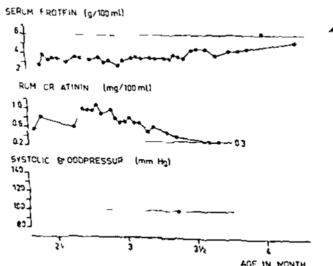


Fig 2 Blood pressure and erum concentrations of protein and tru creatinine Cholesterol 392 mg/100 ml at 2 / months and 222 at 6 months

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CASE REPORT

COINCIDENCE OF CONGENITAL TOXOPLASMOSIS AND ACUTE NEPHRITIS WITH NEPHROTIC SYNDROME

BIRGITTA WICKBOM and JAN WINBERG

From the Department of Paediatrics St Goran s Hospital Karolinska Institute and the Sachsska Children s Hospital Stockholm Sweden

We report here the simultaneous occurrence of congenital toxoplasmosis and acute nephritis with nephrotic syndrome in a 10 week old boy

CASE REPORT

Case history R E 68 07 06 boy Second child of healthy parents delivery normal birth weight 3 200 g Thrived the first few weeks of life

History and findings related to nephritis

Screaming attacks and failure to thrive at 6 to 7 weeks of age Admitted at 10 weeks with generalized pitting oedema and abdominal petechiae No fever no signs of acute inf ction The condition deteriorated rapidly accumulation of oedema and ascites arterial hypertension cardiac and respiratory insufficiency sluggishness proceeding to stupor No excretion of dye on intravenous pyelography The severe condition precluded a renal biopsy No streptococci were isolated ASO titre 25 No antinuclear factor demonstrated Total complement in serum 3–4 weeks after onset was 151 units (control serum 200 ± 50) Cholesterol 392 mg/100 ml Treatment with prednisolon diuretics rauwolfia hydralazine digitalis albumin and blood Findings at admission treatment and course are shown in Figs 1 and 2 At the age of 13 months the systolic blood pressure was 80 mmHg A pyelography was normal creatinine clearance was 90 ml/min/1 73 m and the renal concentrating capacity 1 020 mOsm/l At the last check up at the age of 2 5 years the patient was in good health

History and findings related to toxoplasmosis

Mother The mother had a longlasting fever with lymphadenopathy during the 7th and 8th months of pregnancy The serologic findings of mother and child are given in Table 1 The titres suggest a fairly recent infection in the mother Wasserman reaction negative

Child There was no marked lymphadenopathy thrombocytes 240–115 000 A chorioretinitis was observed 1 week after admission the age of the lesion being uncertain Later a moderate micro-ophthalmia and squint was obvious No intracranial calcifications

The toxoplasma antibody titres in the child rose rather late (*Table 1*) Complement fixation tests were negative against rubella cytomegalic virus entero viruses and adenovirus The mycoplasma antibody titre was 1/32 when investigated about 3 weeks after the onset of the renal disease The mother had a mycoplasma titre of 1/8 The immunoglobulin levels were IgG and IgA 30 and IgM 40 mg/100 ml 3 years later the values were respectively 820 48 and 43 mg/100 ml (low normal values)

COMMENT

The diagnosis of a toxoplasma infection probably congenital as well as of an acute nephritis with a nephrotic syndrome seem to be well founded Congenital toxoplasmosis appears at a rate of 0 05–0 1‰ (4) in Sweden i e in 5–10 cases per year Acute nephritis during the first few months of life is very rare Their simultaneous appearance therefore raises questions about a cause and effect relationship In evaluating this possibility the following points merit some interest Another congenital infection syphilis may in rare instances induce a similar renal disease (7) and another protozoal antigen *Plasmodium malariae* may induce a nephrotic syndrome (2 8) The third

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CASE REPORT

TURNER S SYNDROME IN ONE OF MONOZYGOTIC TWINS WITH MOSAICISM

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Twenty three cases of chromosome abnormality associated with monozygotic twinning have recently been listed by Nielsen (4) In eleven of these both siblings were of abnormal phenotype and chromosome complement In five cases one of the twins had primary trisomy mongolism the other being normal and in six of the remaining cases one twin showed stigmata of Turner s syndrome while the other twin was phenotypically normal The chromosome findings were heterogeneous in these cases and included two cases of twins with 45 X/46 XX mosaicism in the blood

This paper presents a further case of monozygotic although phenotypically dissimilar twins both of whom showed 45 X/46 XX mosaicism on blood culture

CASE HISTORY

Twin girl were born to a primigravida aged 29 years after an uneventful pregnancy lasting 37 weeks

Twin 1 weighing 2 000 g was delivered by the vertex and had an Apgar score of 5 at one minute Twin 2 weighing 1 680 g was delivered 10 min later by the breach with forceps to the after coming head Her Apgar score was 3 at one minute and she required intubation and artificial respiration for four minutes There was a sin le placenta

Both babies were nursed in the special care unit becau e of prematurity Their maturity on the basis of the Dubowitz score at birth was asses ed at 35 weeks

Twin 1 had most of the somatic stigmata of Turn er s syndrome including gross o dema particularly of the face and extremities with marked anterior and posterior cervical skin folds The femoral pulses were palpable and no heart murmur was heard The neonatal course was uneventful and she was discharged at the age of five weeks The oedema gradually de creased but persisted on the dorsa of the hands and feet and the forehead

Twin 2 was an apparently normal premature female infant with no evidence suggestive of Turner s syndrome During the first 24 hours she developed an aspiration pneumonia but responded well to the usual measures Her further stay in the Special Care Unit was complicated by a l ft inguinal hernia which was repaired

Both infants were followed at regular intervals and at the age of one year were noted to have the features listed in Table 1 Twin 1 continued to show the char acteri ti features of Turner s syndrome while Twin 2 was phenotypically normal (Fig 1)

Detailed analysis of blood groups revealed that these were identical (Table 2) The finger prints how ever were dissimilar (Table 5) Twin 1 having a high dermal ridge count compatible with Turner s syn drome

Cytological studies

Buccal smears were taken from each twin on two separate occasions and stained with cresyl fast violet aceate 120 nuclei being examined from each sample Twin 1 was found to be sex chromatin negative Twin 2 was sex chromatin positive 20% of the nuclei hav ing bodies in each sample Sex chromatin studies on cultured fibroblasts showed Twin 1 to be sex chromatin negative and Twin 2 to be sex chromatin positive in both tissues the counts for Twin 2 fell within the exp cted normal female range

Samples of peripheral blood were taken on two oc

Table 1 *Serologic tests of toxoplasmosis in mother and child*

	Child		Mother	
Months after delivery	Dye test	Complement fixation test	Dye test	Complement fixation test
2 1/4	1/250	<1/7 5	—	—
4	1/1250	1/15	1/1250	1/30
4 3/4	1/1250[a]	1/30	1/6250	1/30
41	1/250	—	—	—

[a] All in 7S fraction

of congenital toxoplasmosis—as well as of other congenital infections—is obscure but may be other signs of pathological immune reactions

If the toxoplasma infection was of pathogenetic importance for the renal disease one may ask why similar observations have not been made earlier It should then be pointed out that also in congenital syphilis clinically apparent nephritis is rare (7) In this context the following points merit some interest A late increase in the toxoplasma antibody titre was observed in this patient This may be a consequence of the renal disease either due to urinary loss or to antibody trapping in glomeruli, which has been shown to explain low serum antibody titres in mice with neonatal LCM virus infection (6) A third possibility would be a disturbance of the immunologic homeostasis as seen in some cases of congenital rubella (5) The extremely low IgG level in our patient is worth noticing This might be of pathogenetic importance since the induction of antigen antibody complex nephritis presupposes antigen excess (1) The rare appearance of nephritis in congenital toxoplasmosis and syphilis could be explained by the rare appearance of another event for example an immune defect

SUMMARY

A ten week-old boy with congenital toxoplasmosis fell ill with a severe nephritis with nephrotic syndrome from which he has recovered completely The possibility of an immune complex nephritis induced by Toxoplasma is discussed

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Key words Nephritis nephrotic syndrome toxoplasmosis

Table 2 *Blood groups of the twins and their parents*

Wife	O CDe/cDE	kk	$MNssP_1$	Le^{a-b-}	Wr^{a-}	Lu^{a-}	Fy^{a-}	Jk^{a+b+}
Husband	A_1 cDE/cde	kk	MNSP	Le^{a-b+}	Wr^{a-}	Lu^{a-}	Fy^{a+}	Jk^{a+b+}
Twin 1	A CDe/cDE	kk	MMSP	Le^{a-b-}	Wr^{a-}	Lu^{a-}	Fy^{a+}	Jk^{a+b+}
Twin 2	A CDe/cDE	kk	$MMSP_2$	Le^{a-b-}	Wr^{a-}	Lu^{a-}	Fy^{a+}	Jk^{a+b+}

Table 3 *Results of cytogenetic studies*

Twin I				
Chromosome number	44	45	46	47
Blood 1 (2 days)	—	21	9	—
Blood 2 (6½ months)	—	38	30	—
Skin (1 month)	—	23	—	—
Buccal smear	Sex chromatin negative			
Twin 2				
Chromosome number	44	45	46	47
Blood 1 (5 weeks)	—	20	3	—
Blood 2 (6½ months)	—	31	32	—
Skin (1 month)	—	—	30	—
Buccal smear	Sex-chromatin positive (20)			

anaphase lag has been followed by delayed separation of the twins with resulting inequalities in the distribution of the abnormal cell lines between the twins There may also be variation of distribution of cell lines between tissues and the severity of effect may depend on the relative distribution of the abnormal cell line These events could account for the chromosome distributions in the present case and in those described by Edwards (2) and Ross (5)

In the present pair of twins initial blood culture showed both twins to have 45 X/46 XX mosaicism Further culture at seven months of age confirmed this although an increase in the number of normal cells was observed in both twins However both skin culture and buccal smear were different in the two twins In the sibling with overt Turner s syndrome only 45 X cells were found in the skin and the buccal smear was negative In the unaffected child only 46 XX cells were found in the skin and the buccal smear was positive

This case therefore is the only one to date in which mosaicism has not been found outside the blood It presents the possibility that the mosaicism in the blood of both babies could be the result of a reciprocal transplant across a common placenta If this occurred very early in foetal life the blood mixture would remain as a permanent feature of the twins

Blood chaemerism would also provide a simple explanation in the other cases discussed where disparity of phenotype was accompanied by similarity of blood cell chromosome complement Blood cell exchange could occur whether or not the other tissues proved the twins to be true mosaics In cases where there is the possibility of a common placenta blood culture would seem to be the least useful test

Table 4 *Comparative cytogenetic findings in four monozygotic twins*

Tissue	Mikkelsen et al Twin I (*)	Mikkelsen et al Twin II ( )	Edwards et al Twin I (*)	Edwards et al Twin II ( )	Present paper Twin I ( )	Present paper Twin II ( )		Ross et al Twin I (*)	Ross et al Twin II ( )
Buccal smear	0	46	0	Positive	0	Positive		7–11 + 2 double	24–26 + 6 double
Blood									
X0	30	34	35	36	70	87	X0	80	83
XX	66	67	65	64	30	13	XXX	20	17
Fibroblast									
X0	85	42	Nil	Nil	100	0	X0	95	0
XX	15	50	Nil	Nil	0	100	XXX	5	100

Table 1 *Clinical features at one year of age*

	Twin 1	Twin 2
Height	72 cm	72 cm
Weight	8 9 kg	8 8 kg
Mental development	*Normal*	Normal
Webbing of neck	+ +	–
Oedema of hands and feet	+ +	–
Abnormal facies	+	–
Femoral pulses	Present	Present
Heart	No cardiomegaly *no murmurs*	No cardiomegaly no murmurs

casions and chromosome preparations were made using a micromethod A skin sample was also *taken* from each twin The results of the chromosome analy *es from the* e tissues are given in Table 3

On skin culture Twin 1 was X0 and Twin 2 was XX but on blood culture both twins on each occa *sion showed 45 X/46 XX* mosaici m The relative proportion of XX cells increa ed with time In *Twin* 2 this change was significant (Contingency $\chi^2_{(1)}$ = 8 45 1° *P* 0 1) whereas in Twin 1 it was not significant (Contingency $\chi^2_{(1)}$ = 1 19)

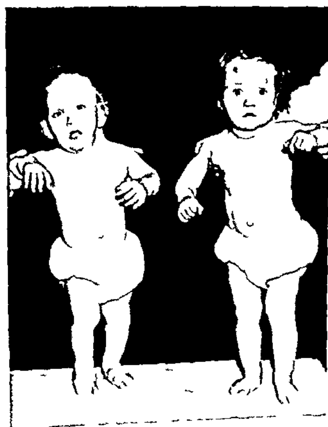


Fig 1 Infants at age of 13 months *(Left)* Turner s phenotype showing lymphoedema of hands and feet typical facies and webbing of neck *(Right)* Normal" sibling

DISCUSSION

A striking feature of these twins was the difference in their phenotype Such disparity between monozygotic twins has been reported previously, but in only three of these cases have both twins shared a common blood chromosome complement Mikkelsen et al (3) described a pair of apparently monozygotic twins that were phenotypically heterogeneous. One sibling showed characteristic Turner s syndrome with severe retardation and died following repeated episodes of pneumonia The other sibling was clinically normal Buccal smear examination revealed that the child with features of Turner s syndrome was sex-chromatin negative while the normal child was sex chromatin positive Blood culture showed 45 X/46,XX mosaicism in both twins This result was confirmed on skin culture

Edwards et al (2) also described apparently monozygotic twins of different phenotype in whom the blood cells were 45 X/46 XX mosaics The twin with features of Turner s syndrome was sex chromatin negative while the normal twin was sex-chromatin positive Chromosome analysis of the fibroblasts was not possible

Ross et al (5) reported presumptive monozygotic twins discordant for gonadal dysgenesis In both cases the twins had sex chromosome mosaicism 45 X/47 XXX in the blood cells A similar karyotype was found in the fibroblasts of the twin with gonadal dysgenesis but the second twin had only 47,XXX cells in the skin The affected child had a low count on her buccal smear but the second twin had a normal count Both had a small number of double bodies in their buccal smears

A summary of the findings in these three cases is given in Table 4

It would appear that two factors affect the distribution of cell lines between twins and between tissues within a twin The first factor is stage in zygote development at which anaphase lag takes place The second factor is the time at which separation of the twins occurs Mikkelsen (3) has suggested in her case that early

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CASE REPORT

COR TRIATRIATUM

A report of two cases

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WIGHER MORTENSSON and ALF RAUSING

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Cor triatriatum is characterized by pulmonary veins opening into a supernumerary left atrium located proximal to the proper left atrium The supernumerary atrium represents the persistent dilated common pulmonary vein The proximal and distal atria communicate through a narrow opening A normal mitral orifice provides further communication to the left ventricle (Fig 1) The diagnosis is difficult to establish in vivo and requires angiocardiography and catheterization

EMBRYOLOGY

Classic cor triatriatum is clearly defined but the condition varies according to the development of the pulmonary veins The lungs develop from the upper part of the digestive tract and consequently they receive their blood supply from the splanchnic plexus and their venous drainage empties into abdominal visceral veins A diverticulum—the common pulmonary vein—develops from that part of the sino atrial region which subsequently becomes the left atrium Later the common pulmonary vein gets in direct connection with the pulmonary veins which at this time are four in number The common pulmonary vein is then incorporated into the dorsal wall of the left atrium and the four pulmonary veins drain directly into the left atrium Cor triatriatum appears to develop as a result of deficient absorption of the common pulmonary vein into the left atrium According to van Praagh et al (14) this occurs because of entrapment of the left atrial ostium of the common pulmonary vein by tissue of the right horn of the sinus venosus from which septum primum develops leading to failure of incorporation of the common pulmonary vein into the left atrium during the fifth embryonic week

There are variations of this classic form and Niwayama (12) and Gasul (4) have given a classification of these according to the size of the opening in the diaphragm Other variations (5) and cor triatriatum dexter have also been reported in the literature (15)

DIAGNOSIS

The symptoms are caused by elevated pressure in the pulmonary circulation often leading to congestive heart failure The age at which symptoms develop depends on the size of the opening between the two left atria and usually occurs in the first year of life If the orifice exceeds 3 or even 6 mm in diameter this

Table 5 *Dermatoglyphic studies*

		V	IV	III	II	I	Total
Finger tips dermal ridge counts							
Twin 1	R	0/12	12/6	0/13	14/0	0/7	145
	L	12/10	6/9	11/9	11/13	0/0	
Twin 2	R	17/17	20/11	16/16	22/0	0/17	266
	L	15/19	20/9	0/16	20/19	0/17	
Palms a-b ridge count							
		Left	Right				
Twin 1		36	36				
Twin 2		36	33				
Maximal *atd* angle							
Twin 1		84	50				
Twin 2		55	90				

to perform Only by the culture of other tissues, including ovarian tissue might the definitive chromosome complement of such twins be resolved

SUMMARY

Cytological studies in monozygotic twins are presented One infant showed characteristic features of Turner s syndrome while the other was phenotypically normal Culture of blood cells revealed 45 X 46 XX mosaicism in both subjects The phenotypically normal twin was sex chromatin positive and showed XX cells only on skin culture, whereas the infant with Turner s syndrome was chromatin negative with only X0 cells on skin culture The significance of these findings is discussed

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Key words Turner s syndrome monozygotic twins mosaicism



Fig 3 (*a* and *b*) Case E. F Enlargement of the right atrium and ventricle The large left atrium bulges backwards The pulmonary veins are dilated because of obstruction to the venous drainage

and the birth weight was 3 860 g Feeding difficulties occurred at the age of 1 months somewhat later followed by symptoms of congestive heart failure Auscultation disclosed gallop rhythm The liver reached 2 cm below the costal arch ECG showed signs of right ventricular hypertrophy and tall broad P waves (Fig 2 *a*) Inverted T waves were recorded over the left precordium At roentgen examination pulmonary venous congestion was found (Fig 3 *a* and *b*) *The patient was catheterized* and elevated pressure was found in the right ventricle and the pulmonary artery but no shunt (Table 1) Angiocardiography showed enlargement of the right ventricle *pulmonary artery* and the left atrium The *left ventricle* was normal (Fig 4) The left atrium could *not* be entered A membrane in the left atrium was observed but was not thought to be the cause of the symptoms The inverted T waves over the *left precordium were interpreted as signs of a focal* myocardial lesion An erroneous diagnosis of acute myocarditis was made and the patient received only anticongestive treatment The conditions deteriorated and the patient died one month after the initial admission

Autopsy revealed that the heart was enlarged (57 g 25 g being *normal for that age*) *The right ventricle* was hypertrophic and the right atrium severely dilated The pulmonary artery was also dilated The left atrium was divided in two compartments by a funnel shaped membrane in which there was a central opening barely 2 mm wide The four pulmonary veins opened into the proximal atrium—the common pulmonary vein The distal left atrium had a normal endocardium The foramen ovale opened into the distal atrium from which a normal auricular appendix projected The mitral valves were somewhat hypoplastic with gelatinous reddish grey deposits The coronary vessels and myocardium displayed no *changes The ductus arteriosus was closed*

Microscopic examination showed fibromuscular

Table 1 *Results of catheterization in two cases of cor triatriatum*

	Case E F				Case A K	
	Pressure					
	Syst	Diast	Average	O_2-saturation	Pressure (Syst)	O_2-saturation
Superior v cava				43		43
Right atrium			5	50		52 49
Inferior v cava				48		66
Right ventricle	82	2 6		50	95	49
Pulmonary artery	80	22	50	52		
Left ventricle	8[illegible]	1 10		96		
Aorta	76	30	49	95		

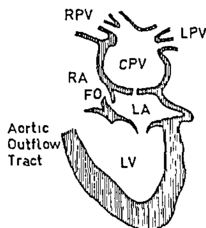


Fig 1 Schematic illustration of the anatomic relationships in cor triatriatum *RPV* right pulmonary veins *LPV* left pulmonary veins *CPV* common pulmonary vein chamber or proximal left atrium *RA* right atrium *FO* foramen ovale *LA* true distal left atrial chamber *LV* left ventricle

lesion will however present clinical signs in the third or fourth decades of life (10 13) The second sound is occasionally split, a systolic murmur is common and there is occasionally a mid diastolic murmur at the lower border of the sternum ECG shows right axis deviation right ventricular hypertrophy and tall, peaked P waves Radiological examination shows enlargement of the left atrium and the right ventricle Catheterization discloses varying degree of pulmonary hypertension, with an elevation of the pulmonary capillary venous pressure In a few cases it has been possible to enter the true left atrium, where normal pressure was found in contrast to the elevated pulmonary capillary venous pressure Angiocardiography is necessary to confirm the diagnosis

In addition to mitral stenosis the following congenital anomalies should be considered in the differential diagnosis subvalvular congenital mitral stenosis a complex of supravalvular ring of left atrium parachute mitral valve subaortic stenosis and coarctation of aorta supravalvular stenotic ring and obstruction to the pulmonary veins with normal or anomalous drainage

CASE REPORTS

E F female born 1968 The mother had epilepsy and was treated with phenobarbital and diphenyl hydantoin during the pregnancy Delivery was normal

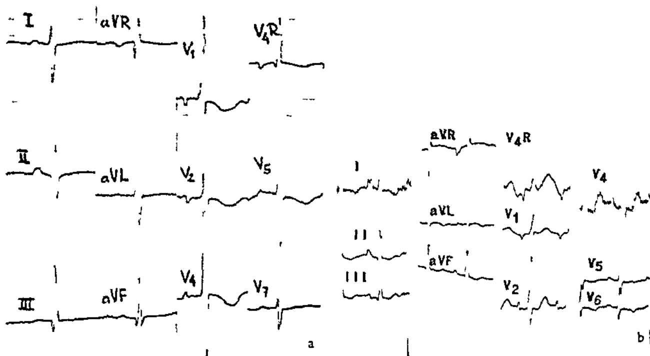


Fig 2 (*a*) ECG Case E F Demonstrating signs of left atrial enlargement and right ventricular hypertrophy (*b*) ECG Case A K Demonstrating double peaked P in lead I and negative P waves in aVR and V R and signs of right ventricular hypertrophy

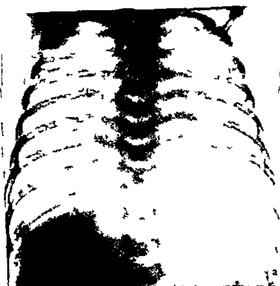




Fig 3 (*a* and *b*) Case E F Enlargement of the right atrium and ventricle The large left atrium bulges backwards The pulmonary veins are dilated because of obstruction to the venous drainage

and the birth weight was *3 860 g* *Feeding difficulties* occurred at the age of 1 ½ months somewhat later followed by symptoms of congestive heart failure Auscultation disclosed gallop rhythm The liver reached 2 cm below the costal arch ECG showed signs of right ventricular hypertrophy and tall broad P waves (*Fig* 2 *a*) *Inverted T waves* were recorded over the left precordium At roentgen examination *pulmonary venous congestion was found* (*Fig 3 a* and *b*) The patient was catheterized and elevated pressure was found in the right ventricle and the pulmonary artery but no shunt (Table 1) Angiocardiography showed enlargement of the right ventricle pulmonary artery and the left atrium The left ventricle was normal (Fig. 4) The left atrium could not be entered A membrane in the left atrium was observed but was not thought to be the cause of the symptoms The inverted T waves over the left precordium were interpreted as signs of a focal myocardial lesion An erroneous diagnosis of acute *myocarditis* was made and the patient received only anticongestive treatment The conditions deteriorated and the patient died one month after the initial admission

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Pulmonary artery	80	22	50	52		
Left ventricle	82	1/10		96		
Aorta	76	30	49	95		



Fig 4 Case F F Contrast injection into the right ventricle In the levogram a diaphragm (between the arrows) is seen dividing the left atrium into a small part to the left—the true distal left atrium and a large part to the right—the persistent common pulmonary vein The pulmonary veins drain into the latter

the third left intercostal space were noted ECG showed left atrial enlargement right ventricular hypertrophy and inverted T waves over the left precordium (Fig 2 *b*) At roentgen examination pulmonary congestion was found Angiocardiography showed enlargement of the right ventricle and the left atrium The patient was catheterized and an elevated pressure was found in the right ventricle but no shunt (Table I) The pulmonary artery was not reached Angiocardiography disclosed a membrane in the left atrium

The patient was in poor condition and was immediately taken to surgery During the operation performed without cardiopulmonary bypass a severely distended left atrium was found and on its outside there was a groove corresponding to the membrane The membrane was divided bluntly Ventricular fibrillation cardiac arrest and atrioventricular block alternated for 35 minutes making heart massage defibrillation and 20 hours of post operative artificial respiration necessary

The patient was re-examined at the age of 2 years Her general condition was good Her weight was 11 3 kg and psychomotor development was normal Right ventricular hypertrophy was no longer apparent in the electrocardiogram

tissue in the membrane in the left atrium Normal endocardium covered the distal left atrium The wall of the proximal left atrium was covered by thick embryonic connective tissue built up of star shaped interconnected cells in an abundant metachromatically stainable matrix with a few connective tissue fibrils The cells were to a certain extent arranged parallel to the surface and the structure was reminiscent of a vascular wall but was much thicker than venous intima It contained no elastic fibrils (Fig 5) The deposits on the mitral valves also displayed a myxomatous connective tissue (Fig 6) There were no histological changes in the myocardium

The lungs weighed almost twice as much as normal and displayed multiple peripheral haemorrhages Microscopically the lungs showed intensive congestion The pulmonary arteries had thickened walls with especially pronounced hyperplasia of the intima and fibrin thrombi In certain places arteritis with fibrinoid necrosis and inflammatory cells and bleeding were seen Lymphatic vessels in the lungs were very dilated

No other malformations were found Bacteriological and virological studies of the heart and other organs produced no significant findings

A K female born 1968 Delivery was normal and weight at birth was 4 430 g At the age of 6 months the patient developed infection of the respiratory tract and congestive heart failure The liver extended 4 cm below the costal arch A third sound at the apex and a low pitched mid systolic murmur in



Fig 5 Microscopical appearance of upper surface of membrane between distal left atrium and supernumerary cavity Elastic tissue stain × 80

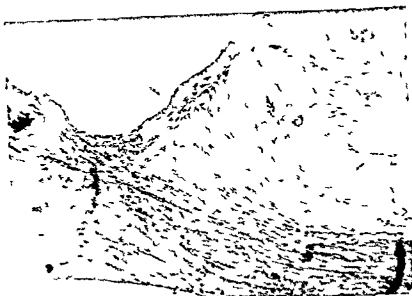


Fig 6 Microscopical appearance of mitral valve demonstrating myxomatous thickening Elastic tissue stain ×90

DISCUSSION

Our two cases displayed the classical picture of the rare anomaly cor triatriatum as it was first described by Church (2) more than 100 years ago But in one of the cases myxomatous deposits were found on the mitral valves Myxomatous connective tissue also covered the wall of the pulmonary vein chamber which is difficult to explain as the valves and the pulmonary veins are laid down separately and at different times No evidence was found to support the view that the valve changes were the residual products of foetal endocarditis

It would appear more likely that both changes were an indication of a mesenchymal abnormality in the cardiopulmonary primordium Cases with similar abnormalities of the mitral valves have been published (9) The myxomatous changes in the valves probably indicate retarded development Hyams & Manion (9) noted that such changes resemble foetal valve tissue In their material comprising nearly 200 cases one case of cor triatriatum was found with this change in the tricuspid valves In our case the mitral orifice was most heavily involved but all valves displayed some thickening The fact that this change is usually found in conjunction with other deformities must be regarded as support for the theory of a mesenchymal defect in the cardiopulmonary primordium

Because of the poor prognosis surgery is generally indicated It is usually performed with cardiopulmonary bypass and a number of methods have been used (1 5 10) they all involve dilatation or removal of the septum that divides the left atria to reduce the obstruction

SUMMARY

Two cases of the unusual malformation cor triatriatum are presented one of which was successfully operated and the other diagnosed post mortem The later case also showed embryonic connective tissue deposits in the proximal left atrium and on the mitral valves

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Fig 4 Case E F Contrast injection into the right ventricle In the levogram a diaphragm (between the arrows) is seen dividing the left atrium into a small part to the left—the true distal left atrium and a large part to the right—the persistent common pulmonary vein The pulmonary veins drain into the latter

the third left intercostal space were noted ECG showed left atrial enlargement right ventricular hypertrophy and inverted T waves over the left precordium (Fig 2 *b*) At roentgen examination pulmonary congestion was found Angiocardiography showed enlargement of the right ventricle and the left atrium The patient was catheterized and an elevated pressure was found in the right ventricle but no shunt (Table 1) The pulmonary artery was not reached Angiocardiography disclosed a membrane in the left atrium

The patient was in poor condition and was immediately taken to surgery During the operation performed without cardiopulmonary bypass a severely distended left atrium was found and on its outside there was a groove corresponding to the membrane The membrane was divided bluntly Ventricular fibrillation cardiac arrest and atrioventricular block alternated for 35 minutes making heart massage defibrillation and 20 hours of post operative artificial respiration necessary

The patient was re-examined at the age of 2 years Her general condition was good Her weight was 11 3 kg and psychomotor development was normal Right ventricular hypertrophy was no longer apparent in the electrocardiogram

tissue in the membrane in the left atrium Normal endocardium covered the distal left atrium The wall of the proximal left atrium was covered by thick embryonic connective tissue built up of star shaped interconnected cells in an abundant metachromatically stainable matrix with a few connective tissue fibrils The cells were to a certain extent arranged parallel to the surface and the structure was reminiscent of a vascular wall but was much thicker than venous intima It contained no elastic fibrils (Fig 5) The deposits on the mitral valves also displayed a myxomatous connective tissue (Fig 6) There were no histological changes in the myocardium

The lungs weighed almost twice as much as normal and displayed multiple peripheral haemorrhages Microscopically the lungs showed intensive congestion The pulmonary arteries had thickened walls with especially pronounced hyperplasia of the intima and fibrin thrombi In certain places arteritis with fibrinoid necrosis and inflammatory cells and bleeding were seen Lymphatic vessels in the lungs were very dilated

No other malformations were found Bacteriological and virological studies of the heart and other organs produced no significant findings

A K female born 1968 Delivery was normal and weight at birth was 4 430 g At the age of 6 /₂ months the patient developed infection of the respiratory tract and congestive heart failure The liver extended 4 cm below the costal arch A third sound at the apex and a low pitched mid systolic murmur in



Fig 5 Microscopical appearance of upper surface of membrane between distal left atrium and supernumerary cavity Elastic tissue stain ×80

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CASE REPORT

GLYCOGEN STORAGE DISEASE TYPE III AND DIABETES MELLITUS

PETER JOHAN MOE, PER ERIK WAALER and ODDVARD GARATUN TJELDSTØ

From the Department of Pediatrics and the Department of Biochemistry University of Bergen Bergen Norway

Increased incidence of diabetes mellitus has been observed in families of patients with glycogen storage disease (GSD) (8 10) A diabetogenic response to glucose tolerance test is also found in most cases of GSD In 1934 Rauh & Zelson (6) published a survey of 8 cases of probable disturbance of glycogen metabolism Glycosuria was present in two of the cases and in one of these originally reported by Wagner & Parnas (13) a true diabetes mellitus developed To our knowledge the combination of an enzymatic proven case of GSD and diabetes mellitus has not previously been reported The purpose of the present paper is to report a 4 1/2 year-old male child with GSD type III who developed diabetes mellitus at the age of 3 years

CASE REPORT

T B is a male child born January 1 1967 There is no known family history of diabetes *mellitus* The parents are first cousins He is the third of 3 children A 12 year-old brother also suffers from GSD while the other brother is healthy The *amylo-1 6-glucosidase* values of erythrocytes from the members of this family have been reported *elsewhere (family* C) (14) Biochemical analysis of liver *biopsy taken* from the brother at the age of 7 years showed high glycogen content and lack of amylo-1 6-*glucosidase* activity (14) Glucose tolerance test performed four times (2.5 g glucose/kg body weight) resulted *in peak* blood sugar of about 160 mg per 100 ml No diabetic symptoms have so far been seen *in* that child

The boy reported here had generalized convulsions from the age of 4 months and he was admitted to the Children's Hospital in Bergen at the age of 4 / months On admission May 1967 the liver edge could be felt about 8 cm below the right costal margin in the mid-clavicular line No other abnormalities were observed on clinical examination

Results of initial laboratory studies were reported as follows hematograms and repeated urine analyses were within normal limits Fasting blood sugars were 77–48–48–37–32–23 mg per 100 ml Only mild acidosis was demonstrated Oral glucose tolerance test (1 3 g per kg body weight) resulted in an increase in blood sugar from a fasting level of 37 to a peak of 155 mg per 100 ml 2 hours after ing tion of glucose (Fig 1) (A relatively low glucose dose was used in glucose tolerance test in our department at that particular time) Epinephrine test showed a completely flat curve EEG tracing during hypoglycemia (32–23 mg per 100 ml) revealed spikes and short attacks of convulsions were observed The parents wanted the child discharged before further studies could be performed and we lost contact with the family for about 2 years

In 1969 we were permitted to withdraw blood from the patient for enzymatic studies Amylo-1 6-glucosidase activity of his erythrocytes was only 0 05 units/g Hb and the glycogen content was markedly increased (Table 1) The parents had been giving the child frequent meals No convulsions had been observed since discharge from the hospital in 1967

Diabetic symptoms were first observed in December *1969* The patient was admitted on January 3 1970 with a history of polyuria polydipsia fatigue and poor appetite the past 3 weeks Four days prior to admission he had a tonsillitis with high fever and was given penicillin

On admission at the age of 3 years the liver edge was still palpable about 8 cm below the right costal margin His height was 100 cm (75th percentile for age) weight 17 kg (75th percentile for *height*) *Fasting* blood sugar was 230 mg per 100 *ml urine sugar* concentration 5 4 g per 100 ml and keton bodies + +

Oral glucose tolerance test (2 0 g per kg body

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Key words Cor triatriatum congenital heart disease

The ratio $A_{\infty}-A_{\infty}$ using iodine spectrum on glycogen isolated from erythrocytes (12) was low when performed three times after the child had developed diabetes mellitus (Table I) A low ratio is found in type III GSD

COMMENTS

GSD is a heterogeneous condition comprising several distinct disorders associated with abnormalities of glycogen metabolism Type III seems to be the most frequent type (2 14) and in this group a defect in the debranching enzyme system activity may be demonstrated in the erythrocytes (1 7 11 14) In the hepatic glycogenoses there is an inability of the liver to release significant amounts of free glucose derived from glycogen stores This results in marked fasting hypoglycemia with a tendency to convulsions and other neurological complications In most cases the tendency to convulsions decreases as the patients grow older This may be due to increased tolerance to hypoglycemia However an improvement with less frequent and severe hypoglycemia has also been demonstrated The tendency toward normoglycemia as the patient grows older is considered to be due to a decrease in insulin output Lockwood et al (3) have demonstrated that insulinopenia is present in adult cases of GSD type I and they suggest that the decreased insulin responsiveness may be a slowly developing process

Increased incidence of diabetes mellitus has been observed in families of patients with GSD In one of the families with GSD type III studied by us (family B 14) there was 12 cases of diabetes Considering this and the relatively large number of reported cases of GSD in the literature it is remarkable that the coexistence of GSD and diabetes mellitus seems to be so infrequent The diagnosis GSD in the case reported by Wagner & Parnas (13) and later by Priesel & Wagner (5) was made without enzymatic studies The patient became truly diabetic at the age of 16 years (5 6) A shrinkage of the liver was demonstrated at that time However in patients with GSD type III who have reached puberty the liver may no longer be enlarged (10)

Typical symptoms and laboratory findings of diabetes mellitus were observed in the reported case at the age of 3 years He tolerated relatively large doses of insulin in spite of the hypersensitivity to insulin observed in hepatic glycogenoses (3 4 8 9) He has now been on insulin therapy for more than a year and there can be no question about the diagnosis of diabetes mellitus No change in amylo-1 6 glucosidase activity or glycogen content of the erythrocytes was demonstrated after insulin therapy was started This finding suggests that the development of diabetes and insulin therapy has had no influence on the defect in his debranching enzyme system activity but his GSD does not seem to cause him any further trouble This of course may be due to the tendency towards normoglycemia and shrinkage of the liver as patients with GSD grow older but it may also be partly due to the hyperglycemia caused by his diabetes mellitus

A disturbance in the pancreatic function and particularly a truly diabetic condition does not seem to be part of the etiology in the hepatic glycogenoses However the role played by the pancreas in the pathogenesis and the course of the disease of the different types of glycogenoses must await further elucidation

SUMMARY

A 4½ year-old male child with type III glycogen storage disease (GSD) is reported He developed symptoms of diabetes mellitus at the age of 3 years and has now been regularly on insulin for more than a year The diabetic condition does not seem to influence the defect of the debranching enzyme activity but the associated insulinopenia seems to prevent fasting hypoglycemia His diabetes is well regulated and he is growing normally A 12 year-old brother with GSD type III has shown no diabetic symptoms

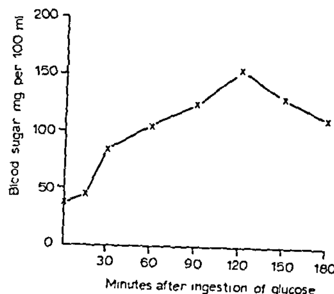


Fig 1 Glucose tolerance test when the patient was 4 ½ months old

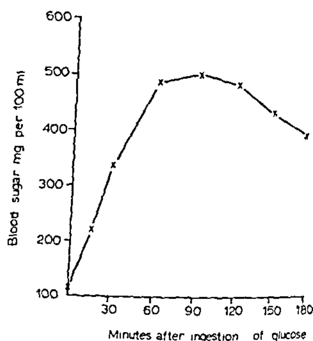


Fig 2 Glucose tolerance test when the patient was 3 years old

weight) resulted in an increase from a fasting level of 115 to a peak of 560 mg per 100 ml at 90 min and 360 mg at 180 min (Fig 2) Plasma insulin activity was 4 μU per ml before and 4 5 and 4 μU per ml 60 and 120 min following ingestion of glucose

The child was poorly adjusted in the hospital All attempts to put him on an adequate diet failed He would only take large quantities of milk yet he required 20 and 28 units of regular insulin during the first 2 days He was discharged on the 6th day without insulin therapy The father was advised to give the patient a diet restricted in carbohydrates and he was seen regularly by one of us (P J M) Recurrence of diabetic symptoms was observed after about 2 months and he was re admitted Fasting blood sugar was 220 mg per 100 ml He refused to eat and it was therefore also impossible at that time to regulate his diabetes in hospital He persistently had glycosuria and ketonuria Fasting blood sugar ranged between 35 and 195 mg per 100 ml After discharge he continued on a daily dosage of 4–6 units NPH insulin until one morning he had a fasting blood sugar of 13 mg per 100 ml Insulin was then discontinued but when the patient was seen 2 months later in July the father had been giving him insulin off and on due to recurrence of diabetic symptoms Weight had decreased from 17 to 14 kg fasting blood sugar was 160 mg per 100 mg Insulin was re instituted and the child has now been on NPH insulin 4–8 units daily for more than a year He had a brief hypoglycemic convulsive attack one morning in January 1971 Fasting blood sugar has later been around 60 mg per 100 ml He is gaining weight and doing well His height is now 106 cm (50th percentile for age) and weight 17 3 kg (50th percentile for height) The liver is palpable only 2 cm below the right costal margin in the medio-clavicular line

Erythrocyte content of amylo-1 6-glucosidase activity has remained low and glycogen content high after he started on insulin (Table 1) Methods for these determinations have been reported previously (14)

Table 1 *Biochemical studies performed on erythrocytes from the patient*

Time of test	Age of patient	Amylo 1 6-glucosidase units/g Hb	Glycogen μg/g Hb	Iodine spectrum A_{460}/A_{390}
January 30 1969	2 years	0 05	224	
January 3 1970[a]	3 years	0 02	510	1 0
January 8 1970[b]	3 years	0 13–0 18	537	0 89
August 21 1970[b]	3 years 7 months	0 05	450	0 89
Normal values		1 7–7 3	< 120	2 2 and 2 5

[a] Diabetes mellitus developed December 1969
[b] After institution of insulin therapy

The ratio A_{460}-A_{380} using iodine spectrum on glycogen isolated from erythrocytes (12) was low when performed three times after the child had developed diabetes mellitus (Table I) A low ratio is found in type III GSD

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GSD is a heterogeneous condition comprising several distinct disorders associated with abnormalities of glycogen metabolism Type III seems to be the most frequent type (2 14) and in this group a defect in the debranching enzyme system activity may be demonstrated in the erythrocytes (1 7 11 14) In the hepatic glycogenoses there is an inability of the liver to release significant amounts of free glucose derived from glycogen stores This results in marked fasting hypoglycemia with a tendency to convulsions and other neurological complications In most cases the tendency to convulsions decreases as the patients grow older This may be due to increased tolerance to hypoglycemia However an improvement with less frequent and severe hypoglycemia has also been demonstrated The tendency toward normoglycemia as the patient grows older is considered to be due to a decrease in insulin output Lockwood et al (3) have demonstrated that insulinopenia is present in adult cases of GSD type I and they suggest that the decreased insulin responsiveness may be a slowly developing process

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A 4 1/ year-old male child with type III glycogen storage disease (GSD) is reported He developed symptoms of diabetes mellitus at the age of 3 years and has now been regularly on insulin for more than a year The diabetic condition does not seem to influence the defect of the debranching enzyme activity but the associated insulinopenia seems to prevent fasting hypoglycemia His diabetes is well regulated and he is growing normally A 12 year-old brother with GSD type III has shown no diabetic symptoms

This seems to be the first reported case of diabetes mellitus in combination with GSD verified by enzyme studies

ACKNOWLEDGEMENT

We wish to thank Ase Larsen Department of Biochemistry for technical assistance

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Key words Glycogen storage disease type III convulsions amylo-1 6-glucosidase activity glycogen content of erythrocytes diabetes mellitus

LETTER TO THE EDITOR

Neonatal tetanus in Iceland

Since early 16th century tetanus was the commonest cause of neonatal death in southern Iceland especially in the Westmann islands off the coast of southern Iceland The same was the case in the islands north of Scotland

During certain periods all newborn children died of tetanus and if possible expecting mothers moved from the Westmann islands and gave birth to their children in other parts of the country

This threat disappeared shortly after the middle of the last century when a Danish physician suspected the infection to be caused by unclean navel dressings and introduced cleaner treatment of the umbilical cord

In May 1960 however a 12 day-old male infant was admitted to the Childrens Department of St Josef Hospital in Reykjavik in a tonic convulsive state with maximal opistotonus He had been delivered at home in the village of Hella on the southern coast north of the Westmann islands The midwife had as usual in Iceland cut the cord very close to the abdomen with scissors after the cord had been tied and dressed with gauze

On admission the umbilicus was incised and cauterised The infant received 40 000 units of tetanus antitoxin i m and 40 000 units i v followed by daily doses of 5 000 units i m for one week In addition penicillin was given He stayed in a tonic condition from May 20 to early July when the convulsions disappeared

Ten years later he was admitted for a minor illness and is now a healthy normal boy in every respect

The clostridium tetani is not a common inhabitant of the soil in Iceland but presumably the millions of seabirds breeding in this part of the country do harbour the bacillus in their intestine

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This seems to be the first reported case of diabetes mellitus in combination with GSD verified by enzyme studies

ACKNOWLEDGEMENT

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Key words Glycogen storage disease type III convulsions amylo-1 6-glucosidase activity glycogen content of erythrocytes diabetes mellitus

and one was above average Three boys showed more or less aggressive tendencies whereas two were antisocial and two emotionally infantile and immature In three of the cases such findings as minimal brain damage disturbed family constellation or rigid educational attitudes were noted

In conclusion the present four cases did not display a common psychopathology However the presence of the numerical chromosome aberration may well predispose to an aggravation of the effect of other noxious influences Therefore boys in whom XYY is found should be carefully observed with special attention paid to their psychic development

Terttu Arajarvi and Tellervo Keinanen *Staying away from school as a symptom of disease*

School phobia is a psychosocial disorder a clinical problem with accompanying social aspects whereas running away from school definitely is a social problem challenging besides the parents and physicians also teachers and school officials and the society as a whole

The material comprises 41 patients who were treated in the Children s Castle of Helsinki over the years 1954–1962 because of staying away from school Follow up inquiries were performed in 1963 and 1969 The percentage of answers received was 98 There was organic aetiology in 10 children The intelligence of the children was on the average level The material was divided into four groups classified as 1) neurotic children 2) those with an immature personality 3) asocial and 4) psychotic children Factors influencing improvement included integrity or defectiveness of the homes improvement of interpersonal relations between the members of the family psychotherapy and the participation of the parents in the treatment

It seems that the homes of children dropping out of school rather need help from the child than offer him help in his difficulties The question arises whether the fear which makes the child drop out of school broadly speaking constitutes a fear of life in the family as a whole It also seems that when the home does not provide a place of security the behavior of the child assumes an asocial nature In the families of neurotic children the parents experienced the difficulty together with the child whereas in the asocial group the homes were already externally more incoherent and especially internally they were exhibiting a rejecting attitude towards the needs of the child

Erkki Jukarainen *Plasma magnesium in the newborn*

The plasma magnesium concentration of 130 neonates was measured by atomic absorption spectrophotometry during the first 5 days of life Thirty of these were normal neonates and thus constituted the control group A total of 1 263 determinations were made The gestational ages varied from 28 to 44 weeks and birth weights from 1 020 to 4 620 g In the control group the mean Mg level ranged from 1 65 to 1 84 mEq/l the highest value occurring at the age of 16–24 hours Significant hypermagnesemia was found at the age of 0–2 hours in the neonates with postnatal asphyxia during the first day in the neonates of diabetic mothers and in those whose mothers had had previous abortions and during the whole observation period of five days in the preterm neonates with gestational age less than 35 weeks Significant hypomagnesemia was found at the age of 16–32 hours in the low birth weight neonates of toxemic mothers and at the age of 56–128 hours in the neonates with birth weight of 4 050–4 620 g The correlations of simultaneously determined serum Ca P and blood glucose to plasma Mg were also investigated

Recently Dancis et al have observed that rat fetus is not parasitic to the mother in Mg deficiency as opposed to the situation in K and Na deficiency When the maternal dietary intake of Mg was very much below normal

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

FINNISH PAEDIATRIC SOCIETY

Meeting Dec 11, 1971

O Simell *The pathogenetic mechanisms in lysinuric protein intolerance*

Lysinuric protein intolerance (LPI, "familial protein intolerance) is a recessively inherited inborn error of aminoacid and ammonia metabolism characterised biochemically by defective renal reabsorption of basic amino acids especially of lysine and by hyperammonaemia and lowered production of urea after amino nitrogen loading 18 cases of LPI are known in Finland

The pathogenetic link between the basic aminoaciduria and hyperammonaemia after protein ingestion has remained obscure Neither the urea cycle enzymes nor glutaminase I of the liver have been found defective in LPI Presently we are working on the hypothesis that the transport of the basic amino acids is deficient in the liver cells as well as in the kidney tubuli resulting in ornithine deficiency at the site of urea synthesis

We have studied the metabolism and the renal tubular reabsorption of arginine and ornithine by constant intravenous infusion technique Glomerular filtration rate was measured with ^{1}Cr EDTA Automatic ion exchange column chromatography was used in the measurement of plasma and urinary aminoacids

A transport maximum was not reached with tubular loads of up to 130 μmole $\times$ min^{-1} $\times$ m^{-2} of arginine and up to 270 μmole $\times$ min^{-1} $\times$ m^{-} ornithine but less was reabsorbed at all levels of the load in the LPI patients than in the controls The constant infusions resulted in about two fold plasma concentration of the infused aminoacid in the 3 LPI patients as compared with 3 controls though the fasting plasma level of these aminoacids in LPI is about $^1/_3$–$^1/$ of the lower limit of normal This constitutes strong evidence for the suggested defect in the transport of arginine and ornithine into the liver cells

Harriet Forsius Ulla Kaski Albert de la Chapelle and Jim Schroder *Is there a common psychopathology in XYY boys?*

It has been repeatedly suggested that males with the karyotype 47 XYY often are tall mentally retarded and antisocial with violent and aggressive destructive behaviour However many authors question this view

The karyotype XYY is quite common the incidence in newborn males being of the order of 1 in 600 Among 145 boys in three small institutions for mentally retarded and/or socially maladjusted boys in Northern Finland one boy with XYY was detected in a pilot screening study using quinacrine mustard fluorescence of buccal mucosa cell interphase nuclei This case together with two other XYY individuals and one XY/XYY mosaic found in paediatric hospital practice underwent a thorough somatic and psychic examination The four patients were between the age of 5 and 15 years Three of the boys had a height above the 50th percentile and two above the 97 5th percentile

Intellectually three of the boys were below

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PEDIATRIC GASTROENTEROLOGY

Meeting in Birmingham April 26–27 1971

HIATUS HERNIA AND GASTRO OESOPHAGEAL INCOMPETENCE

D A W Edwards (London) *The anti reflux mechanism*

In spite of the many interesting physiological and pharmacological properties of the cardiac sphincter it is unlikely that it is the sole or the most important factor in the anti reflux mechanism The compressing force of the circular fibres on the lumen is adequate to assist some other attribute such as the intra abdominal segment of oesophagus when the sphincter is in its normal situation but is inadequate to prevent reflux when herniation has occurred Relaxation of the sphincter on swallowing does not allow reflux or release of a large gastric air bubble in the normal subject The mucosal choke of Chrispin Friedland and Wright the possible effect of the phreno-oesophageal ligament and the application of the "flutter valve hypothesis may each be relevant The biological purpose of the sphincter may be to prime the flutter valve and prevent it being opened by intra gastric pressure produced by contraction of th stomach wall

The maintenance of the lowest few cms of the oesophagus within the abdomen seems the common factor of successful surgical procedures The anti reflux mechanism is probably dependent upon the two cavities of stomach and oesophagus being connected by a short intra abdominal oesophagus closed by the compression of mucosal jelly by the cardiac sphincter and so acting like a "flutter valve

I J Carre (Belfast) *Clinical features and natural history of the partial thoracic stomach (hiatus hernia)*

An outline was given of the clinical features based on an analysis of a personal series of 385 patients The main complaint during early infancy was persistent vomiting from shortly after birth The vomit was usually projectile and copious and often contained traces of altered blood Gastric peristalsis was occasionally seen and in half of these there was also a palpable pyloric tumour though in those subjected to operation no pyloric hypertrophy was found Apart from these patients there was a high incidence of true hypertrophic pyloric stenosis i e 3 6% or ten times the expected frequency Other early features were hunger dehydration failure to thrive constipation and melaena

Vomiting often at night dysphagia anaemia and pulmonary infections were the chief complaints of later childhood In a previous study (1) it was estimated that if given no specific treatment 65% of all clinically affected infants would be symptom free by 2 years 30% would continue to have troublesome symptoms to beyond 4 years before eventually becoming asymptomatic and 5 per cent would develop an oesophageal stricture

Reference

1 Carre I J *Arch Dis Childh 34* 344 1959

A R Chrispin (London) *Radiology in hiatal hernia*

there was an increased resorption rate of the fetuses and the surviving fetuses were small, weak and anemic The analysis of the total fetus demonstrated an increase in Ca concentration this was also evident in the placenta The finding is interesting since it has been argued that the dietary intake of Mg is marginal in the western countries because the major sources of caloric energy (refined sugars fats and grains) are either originally devoid of Mg or lose most of their Mg during the refinement Atherosclerosis has often been connected with dietary Mg deficiency Experimental magnesium deficiency in animals has been demonstrated to produce cardiovascular and renal calcification Further investigations on the possible relation of neonatal Mg deficiency to the later occurrence of atherosclerosis are in progress

Marjatta Koponen *Early physiologic anemia of infants*

The study comprises 180 healthy infants born in the Tampere Central Hospital within a four week period in January–February 1971 *Simultaneous venous and capillary blood* samples were taken as follows immediately after birth, at the age of 12 to 36 hours six days one month two months and three months

In the newborn the mean cord hemoglobin value 161 g/l ± 15 4 agrees with the one presented in the literature, 168 g/l The mean *cord hematocrit, 48% ± 4 6,* is also of the same order of magnitude as the mean value presented in the literature 53% In the new born the mean Hb of capillary samples was 199 6 g/l ± 17 2 and Hcrt 58% ± 5 5 These values are high even higher than those of 1 day old infants This is probably due to the fact that the increment taking place during the first two to three hours of life already shows clearly The venous samples showed a considerable increase of both Hb and Hcrt during the first day of life

At the age of one day venous Hb was 190 g/l ± 18 3 and venous Hcrt 55% ± 5 8, capillary Hb was 196 9 g/l ± 20 8 and capillary Hcrt 57% ± 6 7 After that a gradual decrease up to the age of six days followed and from then on a more rapid decrease which seems to cease at the age of two to three months Hb and Hcrt values measured from the venous blood seem to decrease faster than those from the capillary blood At the age of two months *venous Hb was 111 g/l ± 9 4 and* venous Hcrt 32% ± 2 8, capillary Hb was 121 g/l ± 12 6 and capillary Hcrt 34% ± 3 7 At the age of three months these values measured from the venous blood were the same as at two months and capillary values the same as in venous blood

The lower limit of the Hb seems to be 90 to 95 g/l and of Hcrt 25 to 26%

Olli Koskimies

E Willich (Heidelberg) *Pre and postoperative manometric studies of the gastro-oesophageal junction in hiatus hernia of infants and children*

The intragastric and intraoesophageal pressures above in and below the gastro-oesophageal junction were measured on 46 children (66 occasions) 30 of these estimations were performed preoperatively and 36 postoperatively The measurements were performed using an open water filled polythene tube connected to a Statham transducer and with a pneumograph connected to an electromanometer The children were aged between 3 weeks to 15 years

The results were compared to those of a group of 31 healthy children We found a high pressure in the region of the gastro-oesophageal junction during the third month of life while 60% of the children with hiatus hernia had a reduction in the pressures After operation the mean pressure rose by 1 to 1 1 mmHg 50 of the patients had a reduced pressure There was no statistical difference between the pre and postoperative results In contrast to this the clinical and radiological findings became normal after operation in 80 of the cases

We conclude that (*a*) the diaphragm takes no considerable part in the closing mechanisme of the cardia (*b*) the pressure zone in the gastrooesophageal junction is not a true barrier (*c*) in hiatus hernia there is a higher percentage of pathological pressures in the cardia then in healthy persons (*d*) the pressures after operation on the hiatus hernia do not change significantly compared to the pre operative values and (*e*) following operation the corrected anatomical situation enables the closure of the hiatus regardless of the pressure

GASTRO DUODENAL ULCERATION

R Astley (Birmingham) *Radiological diagnosis*

J D A Robb (Belfast) *Duodenal ulcer in children*

Using strict radiological criteria a diagnosis of duodenal ulcer was made in 49 cases covering the decade 1960–1970 Males predominated 3 1 There was a delay in diagnosis of approximately two years

The principal presenting symptoms were pain vomiting nausea and haematemesis Among subsidiary symptoms a high incidence of dyspepsia was recorded

The pains had no particular pattern in contrast to a clear rhythm of short remissions and short exacerbations of the syndrome

The percentage of fathers mothers and grandparents with peptic ulcer was highly significant There was also a significant increase in blood group 0 in ulcer cases Unequivocal emotional problems were documented in 60% of males and 50 % females

Maximal acid output in response to Pentagastrin was studied in the ulcer group and in those who had failed to satisfy the radiological criteria for inclusion in it Comparison of the mean averages of the two groups did not show a difference of any significance

R H Jackson (Newcastle) *Genetic studies in peptic ulcer in childhood*

This paper reports the results of an attempt to assess the importance of genetic factors in the development of duodenal ulcer in childhood Two approaches were used firstly a family tree has been drawn up of 44 families each containing a child with a duodenal ulcer and the frequency of ulceration among the different degrees of relatives established Secondly blood and saliva samples were taken from propositus and first degree relatives and the genetic markers analysed Similar studies were done on 35 control families matched by sex age and social status with the ulcer family

Blood studies have confirmed an excess of Group 0 among the ulcer cases but not an excess of non secretors No other significant differences between the two groups was found among the genetic markers studied

The oesophageal vestibule is that part of the oesophagus immediately above the stomach In normal infants and young children the oesophageal vestibule lies partly in the thorax partly in the diaphragmatic hiatus and partly in the abdominal cavity The strong fibroelastic phreno oesophageal membrane is attached to the under aspect of the diaphragm and is inserted into the vestibule (1) The vestibule has an inner mucosal layer and an outer smooth muscle layer which has sphincteric characteristics (2, 3)

Radiology is principally concerned with the commoner sliding type of hiatal hernia rather than the para oesophageal type which is rare in infants and children In the sliding type hiatal hernia the vestibule lies, at least part of the time entirely within the thorax Radiologically the vestibule lying in the thorax can be defined as it closes and it may be seen to open with a dry swallow or with crying Numerous thick coarse folds can be seen at the level of the hiatus the edges of which bring the gastric mucosal folds into apposition The column of contrast medium may be arrested in the oesophagus above the closed vestibule

Reflux from the infra diaphragmatic loculus of the stomach into the supra diaphragmatic loculus and oesophagus results in oesophagitis stricture formation oesophageal dilatation and reduced oesophageal motility Such reflux is a consequence of failure to maintain apposition of the mucosal folds within the vestibule which lies entirely within the thorax and at the level of the hiatus which is occupied by the stomach (4, 5)

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5 Chrispin A R & Friedland G W *Ibid* 2? 422 1967

I J Carre (Belfast) *Long term results of postural treatment in children with a partial thoracic stomach (hiatus hernia)*

The results were reported of a long term prospective study of the use of postural therapy in children with a partial thoracic stomach but no stricture All patients studied were nursed throughout the 24 hours in a sitting position of at least 60° Using strict objective assessment criteria the progress of treated patients was compared over a minimum period of four years with the expected outcome as determined from a previous study of the natural history (1)

The value of this form of therapy was confirmed The best results were recorded when treatment was started early e g of 63 patients started on treatment before 3 months of age 53 were symptom free at 1 year and 60 at 2 years all had remained well on subsequent follow up It was emphasised that treatment may need to be continued for many months since the clinical response to therapy is often slow Despite an absence of symptoms a partial thoracic stomach was still radiologically demonstrable in about two thirds of the children examined between 2 and 4 years of age but in only one fifth of those who were between 10 and 15 years at the time of examination

Reference

1 Carre I J *Arch Dis Childh* 34 344 1959

D J Waterston (London) *The treatment of hiatus hernia in infants and children*

Hernia through the diaphragmatic oesophageal hiatus is a common congenital abnormality Over 1 000 cases have been seen at The Hospital for Sick Children Great Ormond Street in the last twenty years The majority of these have presented with symptoms during the first weeks of life

Unlike the pancreatic α amylase the di saccharidases of the brush border membrane sucrase isomaltase and lactase are fully de veloped at birth after normal gestation

As regards protein digestion peptic hydrolysis is probably reduced in the stomach of infants whereas pancreatic proteases are al ready present at the levels of the adult range

Data from the literature are reviewed show ing that all the mucosal peptidase activities studied so far are fully developed at birth Furthermore data are presented showing that hydrolytic activities toward glutaminylproline γ glutamyl and pyrrolidonyl β naphthylami des are also present at birth at normal levels The possible relation of these enzymatic ac tivities to the digestion of gluten proteins was discussed

B A Wharton (London) *Protein nutrition in the newborn baby*

The protein nutrition of a newborn baby may be considered in three stages What are the child s requirements? How may these be translated into a practical feeding schedule? In what way can the adequacy of the feeding schedule be monitored in the individual baby?

The requirements and advisable intakes of protein and aminoacids during the early months of life have been based on nitrogen balances observed changes in body composi tion during development and the actual in takes of healthy babies Unresolved problems in the translation of this information to prac tical feeding regimes have been largely con cerned with the amount of protein to give to low birth weight babies and the qualitative differences between the protein of cows and human milk The protein nutritional status of the individual child can be assessed by growth rate and possibly by plasma albumin and urinary hydroxyproline

The ultimate arbiter in any consideration of infant feeding is survival and the subse quent physical and intellectual development of the child

June K Lloyd (London) *Should babies count calories?*

With the decline in breast feeding the food intake of most infants is governed by paren tal decisions based on advice from various sources Such advice is often dogmatic and tends to ignore nutritional individuality calorie requirements are given as average values defined in relation to body weight During the first few months it is assumed that milk will provide total calorie requirements how ever additional food (usually sugar or cere als) is often given at an early age A recent survey showed that the mean intake of 6 week old infants was 135 kcal/kg/day (range 110–160) and all infants were already receiving non milk supplements (1)

Adequacy of calorie intake is commonly as sessed by weight gain but excessive gain tends to be ignored Artificially fed babies gain weight more rapidly than breast fed babies (1 2) Taitz found about 60% had weights greater than the 90th centile at 6 weeks Ex cessive gain in early life may be associated with obesity in later childhood (3 4) Treat ment of childhood obesity is difficult and the prognosis poor prevention may depend at least in part on the avoidance of giving in fants calorie intakes in excess of individual requirements

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W L Burland & P D Samuel (Glaxo Laboratories Greenford Middlesex) *Meeting the needs the role of industry*

Since its introduction there have been ad vances in techniques for the manufacture of dried cows milk for feeding infants Many modifications and additions have been made but fortification with vitamin D created problems

The family studies have shown marked differences between the two groups with a statistically significant increase in ulcers among relatives of ulcer patients in all degrees of relationship, especially among male relatives of male propositi Calculation of the heritability gives a figure of 0 91, a high result showing that the genetic component in the development of duodenal ulcer in childhood is greater than that in for example congenital pyloric stenosis

INFANT NUTRITION

E Kerpel Fronius (Budapest) *Historical introduction and feeding patterns in Europe*

Most feeding patterns are based on Biedert s century old suggestion to dilute cow s milk and replacing lost calories by fat and carbohydrate Endeavouring safety from enteral infections, acidification or stimulation of the growth of bacterium bifidum in the gut were used With pH 4 5 bacterial growth is inhibited in acidified milk enteral fat loss however, is increasing and the kidneys of young premature infants may not cope with the acid load In most actually used acidified formulas the degree of acidity is less than required for the inhibition of bacterial growth The best promotion of bifidum flora is obtained by adding living strains to special formulas Although B bifidum cultures antagonize specific B coli strains in vitro neither these nor acidified mixtures guarantee convincingly protection from enteral infections Actually 'adapted milks containing less protein and ash than cow s milk, with the addition of vegetable fat and carbohydrate are most widely used Although fat absorption does not reach the level of breast milk in any formula actually recommended all secure growth and development These brilliant results are valid only under good hygienic conditions In under privileged population still breast feeding must be advocated, the second best solution being acidification

E M Widdowson (Cambridge) *Absorption of fat, calcium and vitamin D in the newborn*

Little is known of the mechanisms of absorption of fat, calcium and vitamin D in the new born at a cellular level or how their efficiency differs from those in the adult We know however from balance studies on newborn babies that the ability to absorb fats other than those in breast milk is often low This depends on the fatty acids present in the fat its triglyceride structure and the quantity fed

If the fatty acids in the food remain unabsorbed they combine with calcium in the intestine to form insoluble soaps, which tend to be excreted in the faeces If this goes on to any extent the absorption of calcium is seriously impaired and may be very low even from preparations based on cow s milk The absorption of vitamin D too, is hindered if fat absorption is poor

Premature babies are even less able than full term ones to absorb fats other than that of breast milk and their absorption of calcium from cow s milk preparations may be very low indeed

S Auricchio (Naples) *Intestinal digestion and absorption of carbohydrates and proteins in infants*

The available evidence indicating that the intraluminal α amylolytic digestion of starch in infants is low was discussed This is demonstrated by the low levels of α amylase in intestinal juice as assayed in vitro and by the type of digestion products found in the intestinal juice in vivo following a test meal containing amylopectin Nevertheless measurement of absorption coefficients for wheat tapioca and maize starches indicate that infants absorb almost completely these boiled starches This applies to 30–45 day old and 90–105 day old infants fed with 10 g and 30 g of starch respectively It remains however to be clarified whether the nutritive value of starches is affected by cooking and food processing

degrees The pyloric hypertrophy closely resembled the tumour of human infantile pyloric stenosis Similar ulcers but little muscle hypertrophy were seen in puppies born to an untreated mother who were also given Pentagastrin after birth These findings indicate that prolonged treatment with Pentagastrin may produce duodenal ulceration and pyloric hypertrophy in puppies and that the two lesions may coexist in the same individual However they may also occur separately and it is suggested that they result from different actions of the hormone

K H Schafer (Hamburg) *Spastic hypertrophic pyloric stenosis as embryonal malformation caused by Thalidomide*

From October 1959 until July 1962 about 5 000 infants were born with Thalidomide induced malformations in West Germany

At that time we recognized among the visceral manifestations of Thalidomide embryopathy a high frequency of spastic hypertrophic pyloric stenosis (SHPS) During previous years the frequency of SHPS tended to be from 2 to 4°/₀₀ and during the era of Thalidomide it was 2 5°/₀₀ in children without congenital malformations caused by Thalidomide During the same period 10 cases of SHPS were found in Hamburg among 95 children with congenital malformations caused by Thalidomide According to a representative statistic involving numerous paediatric hospitals of Germany 36 1°/₀₀ equalling 32 cases of SHPS were found among 877 cases of congenital Thalidomide induced malformations. In other words the frequency was ten times higher than in the normal population Statistically this difference is highly significant

Among the visceral Thalidomide induced malformations SHPS is third in frequency following those of the heart and kidneys There is no difference between usual cases of SHPS and those induced by Thalidomide either in clinical or X ray findings or in the clinical course of findings during operation

Nor was there any difference in sex ratio namely 4 male versus 1 female At the moment we are unable to give a definite interpretation of our findings

E Blanche Butler A Holzel & V Miller (Manchester) *Gastric epithelium in the neonate and infant*

Gastric biopsy is not a realistic procedure in babies A method of obtaining material by gastric washing is presented The minute fragments of gastric mucosa obtained are concentrated and processed histologically

In this preliminary study serial sections have been stained for mucopolysaccharides using the simple programme set out in Carleton s Histological Techniques (Fourth Edition) p 205 Sections of neonatal stomach removed at necropsy were submitted to the same stains and it was found that there was no difference in staining pattern of superficial mucosa from pylorus body or cardia of stomach

Gastric mucosa from 48 infants was examined Although there was a typical staining pattern some infants showed an atypical reaction to one or more of these stains The history of these babies showed that this was more likely to happen in ill babies

This is a preliminary study but it is felt that the procedure has a promise as a method of sampling gastric epithelium in infants It will be necessary also to use a more sophisticated staining programme for mucopolysaccharides

L Cathelineau (Paris) *Pepsins in gastric juice of infants and children*

Pepsins in gastric juice were studied in children on a normal mixed diet (first group) and in infants less than 3 months of age on a milk diet (second group) Gastric juices were obtained after histamin stimulation Two types of separation were performed agar gel electrophoresis and column chromatography

Recommended intakes and minimum requirements for energy and essential nutrients are used when formulas and feeding instructions are prepared by us Requirements for optimum growth in preterm infants are ill defined and appropriate diets remain a problem Substitution of butter fat with fats containing shorter chain and polyunsaturated fatty acids results in better absorption but creates problems in manufacture

Neonatal hypocalcaemia probably associated with transient congenital hypoparathyroidism is aggravated by the high phosphorus content of cows milk Hypocalcaemic convulsions are rare but may not be without consequence A limited reduction in the phosphorus content of cows milk is possible Vitamin D and the Ca/P ratio are important

The choice of carbohydrate is limited by availability concern for restricting the osmotic load and a knowledge of available intestinal enzyme activity Amylase and lactase are not fully developed at birth

Folate and iron deficiency occur in preterm infants Dietary folate is inadequate for preterm infants and absorption may be incomplete Iron from fortified milks is insufficient for preterm infants Absorption of dietary iron deserves further attention

FREE PAPERS

D Nussle & R Deleze (Lausanne) *The syndrome of pharyngeal dysphagia inspiratory laryngeal stridor and hiatus hernia in infants*

The frequent association of broncho pulmonary infections, neurological signs and vomiting with inspiratory stridor in infancy prompted us to study systematically this latter disturbance with cinefluorography The act of swallowing as well as the morphology and function of the cardia were studied simultaneously Many cases were also examined by laryngoscopy

80 infants and children under 2 years of age presenting with congenital inspiratory stridor were studied In nearly all cases we found a disturbance in swallowing The movements of the different parts of the pharynx during inspiratory stridor point to a functional defect of the musculature rather than a cartilaginous softening There seems to be a pharyngeal dysfunction responsible for both the stridor and dysphagia

In half the cases the stridor was the only revealing symptom of the swallowing defect In more than half the cases (60%) hiatus hernia with reflux was found

Another survey showed that of 199 children under 2 years of age presenting with swallowing disturbances with or without stridor 135 (68%) had a concomitant hiatus hernia representing 32% of all cases of hiatus hernia in this age group

We must thus stress the frequent association of dysphagia and hiatus hernia often being revealed by inspiratory stridor and the high risk of aspiration in these cases For diagnosis both the act of swallowing and the morphology of the cardia have to be examined

J A Dodge (Belfast) *Experimental production of neonatal pyloric hypertrophy in puppies*

The hormone gastrin stimulates acid and pepsin secretion by the stomach and also stimulates antral motility It has recently been shown to cross the placenta in dogs and probably does so in humans The synthetic preparation Pentagastrin has similar physiological properties to the natural hormone and was administered in depot form to two pregnant bitches for a period of three weeks before their puppies were born Newborn puppies of both these mothers had puloric hypertrophy at birth and in one case there was superficial ulceration in the pyloric canal Others of the offspring were given further doses of depot Pentagastrin for variable periods and some of these developed large duodenal ulcers Muscular hypertrophy was also present in varying

frequent interruptions that sometimes were quite extensive Dilatation of the lymphatics was prominent

The changes observed probably represent a non specific pattern of response of the intes tinal mucosa to injury in this case to restric tions in the supply of calories or protein

N Steiner B Hadorn C Sumida & H Gotze (Bern) *Solubilisation and activation of intes tinal enterokinase by bile acids*

There is good evidence that intestinal entero kinase is a brush border enzyme (1 2) The addition of bile acids to a purified brush bor der preparation of the rat intestine results in solubilisation of brush border bound entero-kinase Concomitantly the activity of the en zyme towards its substrate trypsinogen in creases approximately 5 times depending on the concentration of bile acids used The tau rine and glycine conjugates of deoxycholic but not the conjugates of cholic acid show this effect By adding glycodeoxycholic acid to al ready solubilised enterokinase we could achieve a further activation of the enzyme This would indicate that the increase of ac tivity observed with the bile acids is due not only to the solubilisation but also to an acti vation of the already soluble enzyme The ef fect of bile acids on enterokinase "release from the mucosa was also tested using an iso lated loop of the rat small intestine and per fusing it with a solution of bile acids in vivo The perfusates which contained bile acids had higher enterokinase concentrations This was also observed when whole rat bile was added to the perfusate The possible significance of these experiments is obvious but the results of similar experiments carried out on the in ta t small intestine in vivo must be awaited before the significance of these findings for the digestive process can be evaluated

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J Frenzel & W Plenert (Jena) *The influence of nutrition on the adaptation of acid-base balance in the first days of life*

There are discrepancies between the reports of several authors about the degree of meta bolic acidosis in mature newborns Testing the hypothesis that these discrepancies could be caused most probably by differences in the regime of nutrition we investigated the changes in acid base balance in 212 healthy mature newborns receiving the different re gimes

Actual pH pCO base-excess and standard bicarbonate were measured daily in arterial ised capillary blood by the method of Astrup The detailed statistical evaluation of the re sults led to the following conclusions

1 The quantity and kind of nutrition had no demonstrable influence on acid base bal ance in mature newborns

2 The time of onset of feeding was of real significance to the acid base balance there was regularly found a slight metabolic acido sis in fasting newborns on the second day of life The newborns receiving one of the two early feeding regimes (beginning 4–6 hours after birth) had an almost normal acid base balance already 24 hours after birth

3 These effects of early feeding were not significantly influenced by quantity or quality (water vs human milk)

L Taitz & F Harris (Sheffield) *Accelerated weight gain in artificially fed babies*

Previous studies from the Department of Child Health in Sheffield have shown

(a) That young infants showing excessive weight gain in the neonatal period and early infancy have an increased tendency to obesity in later childhood compared to infants who did not gain weight at an excessive rate (1)

(b) That the incidence of excessive weight gain in the first six weeks of life amongst in fants in Sheffield was 59 % (2)

The present study was undertaken to con firm the high incidence of excessive weight

on DEAE cellulose Peaks obtained by chromatographic elution were further analysed by electrophoresis and submitted to pH 7 25 inactivation

In the first group electrophoresis yielded four bands of enzyme activity Bands 2 and 4 in order of decreasing mobility had much stronger activity than the two other bands In gastric juices of high pH bands 1 and 3 were absent With chromatographic separation only two peaks were demonstrable the first one was the band 4 on electrophoresis, the second one was the band 2 on electrophoresis This second peak was inactivated at pH 7 25, while the first was not

In the second group (infants) acid gastric juices gave the same results as were obtained in older children The chromatographic separation of alkaline gastric juices showed only a very early peak interpreted as the pepsinogen

These preliminary results appear to establish that gastric juice proteolytic enzymes are the same in infants children and even adults, with the reservation of possible non activation of pepsinogens in some young infants

A W Wilkinson (London) & R A McCance (Cambridge) *Some effects of large intestinal resection in newborn pigs*

The whole of the ileum except the last few cm has been removed from 17 large white pigs about 10 days old and weighing about 4 kg Two have reached maturity but not quite full size and one has had three litters

Removing the whole of the jejunum has been much less traumatic The operation has been performed on 7 animals and 4 have grown almost normally and reached their full genetic stature and performance

The terminal ileum and the whole of the large intestine above the lower part of the descending colon have been removed from 11 animals Five have grown rather slowly reached almost a normal size and produced litters satisfactory

The upper end of the jejunum has been anastomosed to the middle of the ileum and the whole of the jejunum and upper ileum left as a blind loop in 20 piglets Not more than 5 of these are likely to reach maturity and they will certainly be small Sexual development is taking place

Diarrhoea has not been a serious problem The digestion of fat has been good It is difficult to find evidence that the parts of the gut left behind hypertrophy in weight or length

O Brunser A Stekel N J Smith & F Monckeberg (Santiago Chile and Seattle USA) *The small intestinal mucosa of malnourished pigs*

The histologic changes of the intestinal mucosa of pigs with marasmus or kwashiorkor were studied

In marasmus the architecture of the mucosa was preserved Mild non specific changes were present and consisted of increased cytoplasmic basophilia in the epithelium at the tip of the villi, which were somewhat blunted The brush border was thin and dark cytoplasmic granules were frequently seen in the supranuclear cytoplasm Studied with the electron microscope the microvilli were short irregularly spaced and sometimes branched. Lysosomes and free ribosomes were increased in numbers Vesicles containing lipoprotein particles were observed in the vicinity of the Golgi apparatus The basement lamella was of normal thickness though sinuous

In kwashiorkor the changes consisted mainly of some blunting of the tips of the villi and absence of scallopings Cytoplasmic basophilia was increased at the tip of the villi the nuclei were formed in somewhat irregular rows and lymphocytes were frequently seen in the intercellular spaces of the epithelium The microvilli were of normal length and some irregularities in their implantation as well as some branching were seen Lysosomes and free ribosomes were increased in the cytoplasm The basement lamella was thin, with

frequent interruptions that sometimes were quite extensive Dilatation of the lymphatics was prominent

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A W Wilkinson (London) & R A McCance (Cambridge) *Some effects of large intestinal resection in new born pigs*

The whole of the ileum except the last few cm has been removed from 17 large white pigs, about 10 days old and weighing about 4 kg Two have reached maturity but not quite full size and one has had three litters

Removing the whole of the jejunum has been much less traumatic The operation has been performed on 7 animals and 4 have grown almost normally and reached their full genetic stature and performance

The terminal ileum and the whole of the large intestine above the lower part of the descending colon have been removed from 11 animals Five have grown rather slowly reached almost a normal size and produced litters satisfactory

The upper end of the jejunum has been anastomosed to the middle of the ileum and the whole of the jejunum and upper ileum left as a blind loop in 20 piglets Not more than 5 of these are likely to reach maturity and they will certainly be small Sexual development is taking place

Diarrhoea has not been a serious problem The digestion of fat has been good It is difficult to find evidence that the parts of the gut left behind hypertrophy in weight or length

O Brunser A Stekel N J Smith & F Monckeberg (Santiago Chile and Seattle USA) *The small intestinal mucosa of malnourished pigs*

The histologic changes of the intestinal mucosa of pigs with marasmus or kwashiorkor were studied

In marasmus the architecture of the mucosa was preserved Mild non specific changes were present and consisted of increased cytoplasmic basophilia in the epithelium at the tip of the villi which were somewhat blunted The brush border was thin and dark cytoplasmic granules were frequently seen in the supranuclear cytoplasm Studied with the electron microscope the microvilli were short irregularly spaced and sometimes branched Lysosomes and free ribosomes were increased in numbers Vesicles containing lipoprotein particles were observed in the vicinity of the Golgi apparatus The basement lamella was of normal thickness though sinuous

In kwashiorkor the changes consisted mainly of some blunting of the tips of the villi and absence of scallopings Cytoplasmic basophilia was increased at the tip of the villi the nuclei were formed in somewhat irregular rows and lymphocytes were frequently seen in the intercellular spaces of the epithelium The microvilli were of normal length and some irregularities in their implantation as well as some branching were seen Lysosomes and free ribosomes were increased in the cytoplasm The basement lamella was thin with

may have a place in the prevention of absorption of ^{90}Sr y children

C Ricour & J Rey (Paris) *Effects of cholestyramine on the intraluminal kinetics of the hydrolysis and micellar solubilization of fats*

In a previous study we have shown that in a continuous intestinal perfusion of fats at the rate of 20 mg/min/m a steady state is reached at the 120th minute The effects of adding cholestyramine to the system are described

A triple lumen tube was used perfusion and aspiration of the substrate were performed through the first and second lumen the third one was utilized for the infusion of a buffer solution at pH 6 7 Cholestyramine in 5 solution was added to the buffer after 180 minutes perfusion and until the 300th minute (final concentration in the mixture perfused 1)

Chelation of the conjugated bile salt molecules by cholestyramine immediately led to a significant reduction in the percentage of lipids solubilized in the aqueous phase the proportion of micellar free fatty acids to total fatty acids actually dropped in a few minutes from 35 (range 25–45) to less than 10 ⁄ the concentration of *remaining bile salts* being below the critical micellar concentration (0 5–2 0 μM/ml) The percentage of fat hydrolysis and lipase activity were not modified

It is concluded that *usual doses* of cholestyramine are sufficient to block bile salts secreted during intestinal fat digestion

D H Shmerling P Leisinger & A Prader (Zurich) *On the familial occurrence of coeliac disease*

Fifteen symptomatic near relatives of 14 out of 108 index patients with proven coeliac disease (CD) were investigated The diagnosis of CD was established by current diagnostic criteria including intestinal biopsy *in all index* patients and in all but one of the relatives (the mother of an index patient who had typical severe CD in childhood and who presents how with bulky stools and anaemia)

In 7 families the following cases of CD were found Two sisters in each of 3 families mother and daughter in 3 and mother and son in 1 further family (4 other families with 4 index patients are related = cousins) Investigations including biopsies of seven relatives of 7 other index patients (3 sisters 2 brothers 1 father and 1 mother) and of the father of 2 affected sisters failed to reveal CD

Because of the occurrence of asymptomatic cases of CD the exact frequency of the disease is difficult to evaluate Using the method of Carter (comparing with the known frequencies of pyloric stenosis and cystic fibrosis) the frequency of CD was calculated and found to be 1 890 (m 1 1133 f 1 430) The frequency of CD in 1st degree relatives was 1 65

D N Challacombe G A Brown S C Black & M H Storrie (Birmingham) *5 hydroxy indoleacetic acid (5HIAA) excretion in the urine of children with coeliac disease*

The excretion of 5HIAA in urine is increased in untreated adults with coeliac disease Increased blood concentrations of 5 hydroxy tryptamine have also been reported When gluten free dietary treatment was given to the affected adults a fall in urinary 5HIAA accompanied the clinical and biochemical remission

This study reports the results of comparable investigations in children Twenty four hour excretions of 5HIAA were measured in 9 children aged 6 months to 2 years 4 months with untreated coeliac disease This initial observation was followed by further measurements of urinary 5HIAA at intervals after the introduction of a gluten free diet

Single 24 hour 5HIAA control investigations were made in 8 children of similar age in hospital with non gastrointestinal disorders Known serotonin containing foods were ex

gain and to assess whether this was associated with increased length It was found that the incidence of excessive weight gain was 49% in this study

The distribution of length of these infants showed a marked tendency to an increased linear size when compared with normal percentile values

The implications of these findings and the possible relationship to local infant feeding practices were discussed

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W Droese & H Stolley (Dortmund) *Protein intake and retention by healthy bottle fed infants in the first six months of life*

The daily nitrogen balances of healthy infants were determined from the first week to the end of the first half year of life The protein contents of the milk formulas were between 1 4 and 2 6 g/100 g From month four onwards 80% of the nitrogen intake was derived from milk protein and 20% of vegetable origin

There is a rapid increase of nitrogen intake as calculated per kg body weight and day during the newborn period The maximum intake was found between weeks seven and ten After the 10th week of life the nitrogen intake is decreasing slowly

The highest nitrogen retention of 40–43% as well as the highest retention in mg/kg/day was found during the second and third week of life with all formulas During the following weeks the nitrogen retention in per cent with the formula containing more protein is lower than with formula with lower protein content However the nitrogen retention in mg/kg/day averages little higher with the formulas containing more protein

These results were compared with data with the nitrogen intake and retention of breast fed infants A rough calculation of body nitrogen content indicates that a protein intake of 2 5–2 7 g per kg body weight and day during month one to three and 2 0–2 5 g during months four to six may be regarded sufficient for normal growth and chemical maturation

H B Valman T Palmer R J K Brown & B Levin (London) *Plasma amino acid levels in small infants on various protein intakes*

D Barltrop T E F Carr G E Harrison & H Shepherd (London and Harwell) *Absorption of dietary strontium using a stable isotope*

The factors affecting the absorption of strontium from the gut of children have been little studied since most workers have been reluctant to employ radioactive isotopes in this age group The purpose of this study was to measure the absorption and retention of dietary strontium using a stable isotope and to measure the selective prevention of absorption by means of an alginate derivative Ten children aged 2–14 years who were hospital in patients were maintained under balance conditions for periods of up to 14 days A stable enriched preparation of ^{8} Sr was added to the diet of 10 children on two occasions both with and without an alginate derivative The isotope content of plasma urine and faecal specimens was determined by measurement of the gamma emission of ^{90}Sr after bombardment with thermal neutrons in one of the Harwell nuclear reactors Comparison of the two periods showed that a fourfold reduction in strontium absorption was achieved by the addition of alginate without significantly affecting calcium balance The excretion of strontium was followed for 14 days in two children who did not receive alginate and it was found that some 20 per cent of the oral dose was retained This figure is similar to that found in previous work in children after pharmacological doses of stable strontium Dietary supplements of alginate derivatives

beta lactoglobulin (BLG) Bovine gamma globulin (BGG) was less frequently positive No IgE antibodies against alpha lactalbumin (ALA) were detected By oral challenge tests in sensitive infants BLG was found to be the most common allergen

Several milk antigens provoked clinical symptoms in some sensitive infants whose sera lacked the corresponding IgE antibody Recently Ishizaka et al and Tada et al reported the possibility of local synthesis of IgE antibodies Thus these antibodies might well be localized in gastrointestinal tract leaving undetectable levels in the serum

Positive results in skin tests were more frequently than those obtained in the RID technique

IgG antibodies usually accompanied those of the IgE type The role of the first in milk allergy is still obscure There is some indirect evidence that they too may be of etiological importance in milk allergy On the other hand they may also possess blocking activity and thus help to prevent allergic symptoms

The existence of IgE antibodies against milk in a celiac patient and in cases of milk aspiration strengthened the idea that milk sensitivity may sometimes be secondary to other diseases

The RID technique was proved to be a useful tool for detecting IgE antibodies to several milk antigens but for diagnostic purposes the value of this test is still in the experimental stage Diagnosis of milk sensitivity still depends on he oral challenge tests as proposed by Goldman et al

T Lindberg (Malmo) N O Berg A Dahlqvist K Lindstrand & A Norden (Lund) *Morphology dipeptidase and disaccharidase activities of small intestinal mucosa in vitamin B_1 and folic acid deficiency*

33 small intestinal biopsies from 24 adult patients with vitamin B_1 and/or folic acid deficiency were analysed with regard to morphology dipeptidase (9 substrates) and disaccharidase activities (5 substrates) 34 adults with histologically normal mucosa and normal serum B_1 and serum folate served as a control group The majority of the untreated patients had short villi (genuine atrophy) A decrease of at least two of the various enzyme activities was found in 20 biopsies The disaccharidases and the peptidyl proline (e g ala pro) peptidase activities were of about the same magnitude in untreated (15 biopsies) as in treated patients (18 biopsies) Unexpectedly the activities against the other dipeptides especially gly leu were definitely lower in the patients treated with vitamin B_1 Five patients with vitamin B_1 deficiency were investigated before and after vitamin B_1 treatment In 4 of them the activities on e g gly leu and ala glu decreased during treatment The activities on ala pro were unchanged and the disaccharidases showed an increase (3 patients) or were unchanged

The study has shown (*a*) that the morphological changes of intestinal mucosa in vitamin B_1 and folic acid deficiency also have biochemical counterparts and (*b*) that certain dipeptidase activities are depressed during treatment with vitamin B_1 The mechanism for this is unknown

J H van de Kamer (Zeist) H A Weijers E A K Wauters & N A Pikaar (Utrecht) *The influence of sterilised milk intake on infants faecal secretion of lysine and cadaverine*

It is known that the sterilisation of milk causes the production of complexes of lysine and lactose (Maillard products) The degree of production of these compounds is dependent on temperature and time of sterilisation

Because it is unknown to what extent these compounds are absorbed or may be toxic (to infants and patients with gastrointestinal disturbances) and into what substances they might be converted by the intestinal flora concerning this problem a preliminary investigation was done in healthy infants

cluded from the diet during the urine collection periods Because of the problems inherent in the collection of timed urine specimens from infants urine creatinine was also measured and 5HIAA excretion was expressed as μg 5HIAA/mg creatinine

Before treatment mean 5HIAA excretion in the children with coeliac disease (25 9 μg 5HIAA/mg creatinine) was significantly greater ($p = < 0\ 005$) than the control mean (13 6 μg 5HIAA/mg creatinine) After gluten withdrawal 5HIAA excretion fell to levels indistinguishable from the controls ($p = < 0\ 1 > 0\ 05$)

The excretion of 5HIAA is proposed as a *diagnostic aid in coeliac disease and a method* for monitoring satisfactory dietary control

E E Lasch R Gruttner & Th Lucking (Hamburg) *The cell mediated immune mechanism and coeliac disease*

Trying to shed some light on the immunological mystery of coeliac disease (1) we recently examined the response of the cell mediated immune mechanism to gluten fraction 3 by using the lymphocyte transformation technique (2) Out of ten children with proved coeliac disease who were investigated by means of this technique four were on a gluten free diet and showed a maximal response to stimulation This response was much weaker in the two children whose diet was inadequate and no response was found in children who were on a normal diet and in whom the disease was shown to be active

Since the transformation by phytohemagglutinin was normal in all the children investigated the negative response to specific stimulation observed in untreated children could not be due to a lymphoreticular dysfunction similar to that found in adult patients

We offer as an explanation of these findings the attraction exerted on the lymphocytes by their specific antigen i e gluten Elimination of gluten from the diet would allow the antigen sensitive lymphocytes to circulate freely in the peripheral blood explaining the positive response found by us in children in remission Introduction of gluten into the diet would on the other hand reestablish this attraction and inhibit the recirculation of the antigen sensitive lymphocytes The intestinal mucosa has been shown to bind gluten, and furthermore, gluten challenge has been shown e g by Shiner & Shmerling (3) to cause infiltration of the mucosa by mononuclear cells which strengthens our postulate

The gluten induced transformation of the lymphocytes would initiate the mucosal lesion by cytotoxic action This mechanism may be *the primary etiological factor in coeliac disease*

References

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B Kletter S Freier Z Noah I Gery & D Levi (Jerusalem) *Evidence for reaginic activity in milk allergy*

The purpose of this investigation is to examine the usefulness of IgE type milk protein antibodies and of prick tests of the skin in the diagnosis of milk allergy

Seventy seven sera of normal and hospitalized infants were tested for IgE antibodies against several milk antigens These antibodies were detected by the radio immunodiffusion (RID) technique using rabbit anti human IgE (Antisera produced in goats cause false positive results)

Immunoglobulin E antibodes were found in 7 out of 26 infants who were suspected of suffering from milk allergy These antibodies were found also in one out of three celiac patients and in two out of five suffering from recurrent aspiration Other sera were negative Most of the positive sera reacted with

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It appeared that the intake of normally sterilised baby milk food resulted in faecal excretion of Maillard products and of lysine and cadaverine the latter substance probable as a result of lysine decarboxylation When lactulose was introduced to the diet the production of cadaverine was suppressed Throughout the trial no clinical disturbances such as diarrhoea, were observed

B McNicholl & B Egan Mitchell (Galway) *Constipation in childhood coeliac disease*

Twelve of 112 children with flat mucosa on biopsy were constipated at some stage before diagnosis Six children aged 6 to 13 months presented with failure to thrive, anorexia vomiting and faecal impaction, 2 had mild intermittent diarrhoea but 4 had never had diarrhoea 1 entered a surgical ward suspected of sub acute destruction Three children had been previously investigated for anaemia growth retardation and constipation with faecal impaction the diagnosis not being made until a second investigation one was referred as Hirschsprung s disease Three children had intermittent diarrhoea and constipation each having laxatives frequently Faecal fat exceeded 4 5 g day in only 3 of 9 children where the 5 day test was done at the time of biopsy daily fat intake ranging from 35 g/day under 1 year to 65 g+in older children Almost 11 % of our cases had constipation 3 5 % did not have diarrhoea

J Jos & C Griscelli (Paris) *Immunoglobulin levels in children with coeliac disease*

A radial diffusion plate method was used to quantitate serum levels of IgA IgM and IgG in 50 children with coeliac disease 19 un treated or in relapse and 31 after treatment with gluten free diet for more than one year Blood samples and small intestinal biopsies were taken simultaneously Immunoglobulin concentrations were determined independently by two laboratories using different anti sera All patients without treatment had a typical flat mucosa In the treated group, the mucosa was normal or slightly impaired

In comparison with healthy children of the same age the patients without treatment had significantly raised IgA concentrations ($p < 0\,0001$) In contrast with other investigators we did not find these raised concentrations to be most outspoken in the youngest patients The concentrations of IgM and IgG in this group were within the normal range

In the treated patients without malabsorption and histological lesions IgA concentrations were normal IgM was raised in about 20 % of the cases, but this was not statistically significant The levels of IgG were normal

P Kuitunen E Savilahti J Rapola & J K Visakorpi (Helsinki) *Malabsorption and cow s milk clinical immunological and morphologic response to provocation*

A boy aged 4 months was found to have a malabsorption syndrome with a flat small intestinal mucosa He had received a wheat free cow s milk formula from 2 weeks and an intolerance to cow s milk was suspected Improvement was seen after a change to breast milk 15 weeks later breast milk was gradually replaced by a cow s milk formula

The following responses were obtained

1 The weight gain decelerated 4 weeks after the start of the provocation

2 The faecal fat increased from 3 2 g/day to 10 8 g/day

3 The serum IgA level rose from 18 to 86 mg/100 ml There was a clear rise in the IgA value of the intestinal juice and stool extracts and simultaneously an even higher rise in the levels of IgM The number of IgA containing cells in the lamina propria of jejunal mucosa rose two fold and IgM containing cells three fold during this provocation

4 The intestinal mucosa was flat before and after the provocation but the height of epithelial cells decreased from 27 μm to 21 6 μm In the electron microscope the microvilli were

short wide and occasionally branched at their tips and often fused at their bases The nuclei of the epithelial cells were irregularly shaped and had lost their perpendicular orientation to the epithelial surface The provocation made these changes worse and the number of lysosomes in the upper part of the epithelial cells increased clearly

After 6 weeks of provocation the diet was changed to breast milk again and the above mentioned changes diminished The results suggest that the malabsorption syndrome the immunological findings and the changes in the jejunal mucosa found in some patients clinically diagnosed as cases of cow s milk intolerance are indeed induced by cow s milk

A M Molla & E Eggermont (Louvain) *Pepsin secretion in coeliac disease*

The noxious effect of gluten on the intestinal mucosa of coeliac subjects is still not understood The study of pepsin secretion in coeliac patients seemed important since peptic digestion of gliadin does not result in the formation of N glutamyl peptides capable of rapid cyclisation to N pyrrolidone carboxyl peptides The latter peptides are tentatively accused of being toxic compounds of enzymatically degraded gliadin (Bronstein et al *Clin Chim Acta 14* 141 1966)

After removal of the fasting gastric residue the secretion was studied by continuous collection of the gastric juice for one hour in 15 minute fractions Subsequently the gastric secretions were collected for another similar period but after the S C injection of Pentagastrin (6 μg per kg) pH volume and peptic activity assayed with the use of the synthetic substrate N acetyl L phenylalanyl L-diiodotyrosine of each fraction were measured

The results obtained from 7 control subjects and 4 coeliac patients aged between 6 months and 2 years are shown in the table The figures indicate the range of the basal values (B) versus those obtained after stimulation (S)

Subjects	Volume output (ml per hour)		Pepsin output (mUnits per hour)	
	B	S	B	S
Control	8–40	5–51	88–716	170–1467
Coeliac	8–34	13–62	0–908	61–1080

Our data indicate that pepsin secretion is not impaired in coeliac disease

D H Shmerling J C W Forrer & Z Manicova (Zurich) *Estimation of faecal fat excretion using CuSCN as a nonabsorbed continuous marker in infants and children*

A method for the estimation of faecal fat excretion using CuSCN as a non absorbable marker and calculating fat from a single spot stool sample has been described by Dick (*Gut 10* 408–412 and 754–759 1969) The results obtained by the author in adults were in close agreement with those from 3-day stool collections

A similar investigation was conducted in 50 children using 150 mg/m CuSCN orally daily as marker Stools were collected during a 5-day period between carmin red markers and an additional stool at the end of the period served as spot specimen CuSCN was given throughout the collection period Total fatty acids and CuSCN excretion were determined in the 5-day stool collection and in the spot specimen Results were expressed as (1) g faecal fatty acids/day uncorrected (mean of 5 days) (2) g faecal fatty acids/day corrected by adjusting for CuSCN recovery (mean of 5 days) and (3) g faecal fatty acids/day calculated from spot stool

The results are shown in the table

	CuSCN Recovery ( )	of uncorrected faecal fat excretion (2)	(3)
$\bar{X}$	71 8	147 6	153 0
S D	2. 6	46 3	100 2
Range	31 6–140	71 4–280	28 4–600

The differences between column (2) and (3) are statistically not significant The relatively

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3 The serum IgA level rose from 18 to 86 mg/100 ml There was a clear rise in the IgA value of the intestinal juice and stool extracts and simultaneously an even higher rise in the levels of IgM The number of IgA containing cells in the lamina propria of jejunal mucosa rose two fold and IgM containing cells three fold during this provocation

4 The intestinal mucosa was flat before and after the provocation but the height of epithelial cells decreased from 27 μm to 21 6 μm In the electron microscope the microvilli were

short wide and occasionally branched at their tips and often fused at their bases The nuclei of the epithelial cells were irregularly shaped and had lost their perpendicular orientation to the epithelial surface The provocation made these changes worse and the number of lysosomes in the upper part of the epithelial cells increased clearly

After 6 weeks of provocation the diet was changed to breast milk again and the above mentioned changes diminished The results suggest that the malabsorption syndrome the immunological findings and the changes in the jejunal mucosa found in some patients clinically diagnosed as cases of cow s milk intolerance are indeed induced by cow s milk

A M Molla & E Eggermont (Louvain) *Pepsin secretion in coeliac disease*

The noxious effect of gluten on the intestinal mucosa of coeliac subjects is still not understood The study of pepsin secretion in coeliac patients seemed important since peptic digestion of gliadin does not result in the formation of N glutamyl peptides capable of rapid cyclisation to N pyrrolidone carboxyl peptides The latter peptides are tentatively accused of being toxic compounds of enzymatically degraded gliadin (Bronstein et al *Clin Chim Acta 14* 141 1966)

After removal of the fasting gastric residue the secretion was studied by continuous collection of the gastric juice for one hour in 15 minute fractions Subsequently the gastric secretions were collected for another similar period but after the S C injection of Pentagastrin (6 μg per kg) pH volume and peptic activity assayed with the use of the synthetic substrate N acetyl L phenylalanyl L-diiodotyrosine of each fraction were measured

The results obtained from 7 control subjects and 4 coeliac patients aged between 6 months and 2 years are shown in the table The figures indicate the range of the basal values (B) versus those obtained after stimulation (S)

Subjects	Volume output (ml per hour)		Pepsin output (mUnits per hour)	
	B	S	B	S
Control	8–40	5–51	88–716	170–1467
Coeliac	8–34	13–62	0–908	61–1080

Our data indicate that pepsin secretion is not impaired in coeliac disease

D H Shmerling J C W Forrer & Z Manicova (Zurich) *Estimation of faecal fat excretion using CuSCN as a nonabsorbed continuous marker in infants and children*

A method for the estimation of faecal fat excretion using CuSCN as a non absorbable marker and calculating fat from a single spot stool sample has been described by Dick (*Gut 10* 408–412 and 754–759 1969) The results obtained by the author in adults were in close agreement with those from 3-day stool collections

A similar investigation was conducted in 50 children using 150 mg/m CuSCN orally daily as marker Stools were collected during a 5-day period between carmin red markers and an additional stool at the end of the period served as spot specimen CuSCN was given throughout the collection period Total fatty acids and CuSCN excretion were determined in the 5-day stool collection and in the spot specimen Results were expressed as (1) g faecal fatty acids/day uncorrected (mean of 5 days) (2) g faecal fatty acids/day corrected by adjusting for CuSCN recovery (mean of 5 days) and (3) g faecal fatty acids/day calculated from spot stool

The results are shown in the table

	CuSCN Recovery ( )		of uncorrected faecal fat excretion (2)		(3)	
$\bar{X}$	71 8		147 6		153 0	
S D		22 6		46 3		100.2
Range		31 6–140		71 4–280		28 4–600

The differences between column (2) and (3) are statistically not significant The relatively

low CuSCN recovery explains the high values in column (2) and (3) Correspondingly the values calculated from the spot stool indicated steatorrhoea (>3 g/day) in 12 cases in whom the actual faecal fat values were <3 g/day (= false positives) Under the same conditions however the values obtained from the *spot stool were below steatorrhoea level in 3* cases whose actual faecal fat was higher (= false negatives)

Because of the low and very widely scattered range of recoveries of CuSCN, this method does not seem to fulfill the requirements for a reliable test for the determination of faecal fat excretion in childhood

G Zoppi, F Pajno Ferrara, G Andreotti & D Gaburro (Verona) *The influence of starch on pancreatic α amylase in newborns*

The influence of starch on pancreatic α amylase activity has been investigated in 10 premature babies with a gestational age ranging between 32 and 34 weeks and a weight at birth between 2 0 and 2 4 kg

The patients were fed a common skimmed milk in addition five of them received 2 g% of soluble starch and five 2 g% of glucose

Pancreozymin secretin tests have been performed three times i e at birth before the first feeding one week and one month later using a double balloon PVC tube as previously described

The α amylase at one month of life becomes 10 times higher than at birth in patients receiving starch whereas it remains practically unchanged in patients receiving glucose

H B Valman (London) *Late vitamin B_{12} deficiency following resection of the ileum in the neonatal period*

Vitamin B_{12} deficiency developed in a child at puberty after resection of 65 cm of the terminal ileum in the neonatal period In contrast another child who had had a similar length of the ileum removed but with preservation of the terminal 12 cm maintained a normal serum vitamin B_{12} level and absorbed vitamin B_{12} normally All infants who have had a resection of more than 20 cm of the ileum should be given a test of vitamin B_{12} absorption preferably using the whole body counter technique If this is impaired, the serum level should be checked regularly at least until adult life so that treatment can be given before complications arise

G W Meeuwisse

Acta Pædiat Scand 61 507-510 1972

PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting Sept 8 1971

I Møller & H Rist Nielsen *Serumtegretol determinations in the treatment of epileptic disorders in childhood*

The first experience in determining serum carbamazepin or tegretol during treatment of convulsive disorders in children is presented During the first two years 287 analyses were made with a spectrophotometric method The majority of the determined concentrations ranged between 0 7 and 0 9 mg/100 ml

Carbamazepin treatment was started in 35 children mostly because of unsatisfactory therapy with other antiepileptic drugs like diphenylhydantoin The dosage was between 10 and 20 mg carbamazepin/day/kg bodyweight A good relationship was found between serum concentration and dosage per weight No advantage was demonstrated in administering the drug by body surface

It is our impression that the prophylactic serumconcentration of carbamazepine is above 0 6 mg/100 ml Side effects were observed among cases with concentrations above 1 2 mg/100 ml mainly decreased appetite slight dizziness and in a single case atactic gait

In 5 cases serumcarbamazepin was determined daily from the commencement of the treatment all were given the same daily dosage An initial rise in concentration was shown in all cases during the first 2 days thereafter a constant much lower and stable level was reached The rise was followed by decreased appetite and dizziness This phenomenon will be dealt with in our further investigations

DISCUSSION

Vesterdal Have you any idea of the reason for the initial rise in serum concentration?

Møller Our present investigations show that the carbamazepin turnover is increased

Brandt How about the specificity of the method?

Rist Nielsen It is good also compared with other methods like thinlayer chromatography We have found no evidence that other products even carbamazepin related substances should influence our results

Vagn Andersen Else Andersen & Bent Friis Hansen *Lymphocytes in neonates Metabolic and morphological investigations*

Incorporation of tritiated thymidine and tritiated cytidine in lymphoid cells from the blood was investigated by means of in vitro incubation and subsequent autoradiography In 6 normal neonates it was found that the initial high concentration of DNA synthesizing cells decreased considerably in the course of the first 2 days of life and then increased again This increase was replaced after 2 further weeks by a gradual decrease to normal adult values The DNA synthesizing cells are approx 15 μ in diameter the nuclei are leptochromatic and the cytoplasm weakly basophilic Cytidine uptake which is an indication of RNA metabolism was assessed by the intensity of marking in the cells and revealed a similar course but with more rapid reactivation following the initial decrease

It is probable but not proven that these findings reflect the activity of the immune apparatus In 6 neonates with bacterial infections higher concentrations of DNA synthesizing cells were observed in the blood than in normal infants A patient with a congenital virus infection had low values

Meeting Oct 13, 1971

E W Flensborg *A syndrome with a Marfan-like phenotype multiple neurinomata of the mucous membranes and endocrine tumours*

A boy aged 13 years was demonstrated with a very unusual appearance of Marfan like type with long slender extremities long fingers and toes In addition he had facial features suggestive of acromegaly with very large prominent lips a large nose and large ears Multiple neurinomata were encountered on the tongue inner side of the cheeks and the lips and an isolated neurinoma was found on the conjunctiva Split lamp investigation revealed increased and thickened nerve fibrils in the corneae The boy had pronounced muscular weakness from early infancy which diminished over the years He had pes cavus X-ray investigation revealed isolated dilatation of the transverse colon (megacolon) Rectal biopsy revealed considerable increase in the number of nerve fibrils in the nerve plexuses of the intestinal wall

This is a case of a rare syndrome which was described first in 1966 by Williams & Pollock These patients develop medullary carcinoma of the thyroid and bilateral phaeochromocytoma frequently at an early stage and in a number of cases parathyroid adenomata develop

Our patient revealed an abnormal reaction to intradermal injection of histamine with absent flare reaction of the same type as in dysautonomia familiaris According to a personal communication from R Gorlin this abnormal histamine reaction indicates that the patient has already a medullary carcinoma of the thyroid

E Lykkegaard Nielsen & Christian Koch *Chronic granulomatous disease*

A patient aged 3 1/ years with chronic granulomatous disease is presented Numerous infections in the form of pneumonias, skin infections otitis, suppurating lymphadenitis and multiple osteomyelitic foci had occurred In vitro investigation revealed that the granulocytes ability to kill bacteria was reduced in the patient and slightly reduced in the mother which confirmed the diagnosis Microscopic examination of a lymph node and tonsillar tissue revealed changes typical of the disease The osteomyelitic changes improved following anti staphylococcal therapy

Five patients with this condition are known in Denmark but the disease is probably under diagnosed The diagnosis is established by microscopic demonstration of characteristic granulomata in the affected tissues and lipoid pigment containing histiocytes and by in vitro demonstration of the defective ability of the granulocytes to kill bacteria or their defective ability to reduce nitroblue tetrazolium

DISCUSSION

Poul Aabel Østergaard *The nitroblue tetrazolium test (NBT test) stimulated with typhus vaccine*

The following investigations were inspired by the demonstration by Grush & Mauer that in healthy children injections of TAB vaccine increase the percentage of NBT positive granulocytes from approximately 7% to approx 40% and also by the investigations by Park & Good on the ability of a specially prepared endotoxin to stimulate reduction of NBT to formazan

A material of 20 healthy children and one boy with chronic granulomatous disease (CGD) took part in this investigation The trial solutions consisted of 1) a standard granulocyte solution 2) a 0 2% NBT solution and 3) commercially available typhus vaccine

The advantages of employing typhus vaccine as stimulator for NBT reduction were the following 1) The vaccine is cheap relatively stable and always easily obtainable 2) The vaccine is as stable a stimulator of NBT reduction as latex and bacterial suspensions 3) The

vaccine causes considerably less granulocyte agglutination than employment of latex and bacterial suspensions 4) Employment of typhus vaccine does not involve the same risk of false positive results as bacterial suspensions Bacteria which have undergone phagocytosis are frequently strongly stained with e g May Giemsa Grunwald so that granulocytes may appear as NBT positive at subsequent microscopic examination 5) On account of the homogeneous nature of the solution changes in staining of the granulocyte suspension in positive reactions to the test from yellow to purple red may be employed for spectrophotometric quantitative analysis of the results

J Helweg Larsen *Larsens syndrome* (not received)

DISCUSSION

Niels Brandstrup *Larsen s syndrome*

We recently had the opportunity to observe a girl aged 4 years who was admitted for observation for a malformation syndrome diagnosed as a variant of Larsen s syndrome Investigation revealed the following abnormalities

1) *Peculiar configuration of the face* with hypertelorism prominent forehead and nose depressed bridge of the nose and a long prominent upper lip 2) *Tendency to dislocation of the lower jaw* which could be pushed forwards and backwards like a drawer The angle between the ramus and the body of the mandible was strikingly large 3) *Submucous cleft palate* and very high palate 4) *Abnormal positioning of the teeth* with inward growth of the teeth in the upper jaw 5) *Hypermobility* in the wrist and metacarpophalangeal joints 6) *The fingers* were short and plump and spindle shaped (in contrast to the long cylindrical fingers described by Larsen) 7) *Chromosome investigation* revealed a single breakage of a chromosome in the 4–5 group but otherwise normal conditions The *mental and motor development* were considered to be normal

O Faerø, J Paulsen & J Rosen *Congenital stridor caused by a cyst in the aryepiglottic region*

Congenital stridor is a characteristic symptom in various pathological conditions of the upper respiratory tract and demands more detailed investigation before the diagnosis of laryngomalacia is made by exclusion

A male infant aged 4 days was admitted on account of feeding difficulties with regurgitation which commenced during the second day of life There was no stridor or cyanosis at birth From the eighth day of life the patient presented increasingly stridorous respiration comprising continual inspiratory stridor and periodic expiratory flapping sound which disappeared in the prone position The voice was slightly hoarse and there was slight subcostal indrawing but no cyanosis

Laryngoscopy revealed a broad based cyst the size of a hazelnut originating from the left aryepiglottic fold and the inner surface of the superior laryngeal cavity This cyst fluttered over the opening of the larynx and caused the stridor The cyst was removed It was found to be thick walled and to contain viscous secretion The histological diagnosis revealed that the cyst wall consisted of respiratory tract mucosa and multilayered squamous epithelium

The patient s symptoms disappeared in the course of 3 days after operation and the condition was entirely satisfactory 8 days after operation On follow up examination 2 months later completely normal conditions were found on laryngoscopy and the infant was thriving

A Rosenklint *Metaphyseal dysostosis (chondrodysplasia metaphysaria)*

The patient was a girl aged 2 years who was admitted for observation for rickets on account of pronounced bowing of the legs

Rickets and other skeletal conditions with alterations in the blood chemistry could be excluded The radiological findings were typical of slight metaphyseal dysostosis Approximately 100 cases of this condition have been de-

Meeting Oct 13 1971

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BOOK REVIEWS

R H Gevers & J H Ruys (ed) *Physiology and pathology in the perinatal period* Leiden University Press, Leiden 1971 199 pp illus D Fl 36—

This book contains the proceedings of a Boerhaave course for postgraduate medical education held in Leiden 1969 During the last decade perinatal medicine has been established as a separate subspeciality and has been the subject of several international congresses and symposia Due to difficulties in reaching the foetus for diagnostic and therapeutic procedures and because of the complicated mechanisms governing the homeostasis of the foeto-placental unit much of our present knowledge of the prenatal patient is based upon work performed by physiologists and biochemists. Thus modern conferences on prenatal medicine try to integrate basal medical disciplines with pediatrics and obstetrics as is the case in the present book

Twelve papers are delivered in the book. The first section deals with various antenatal subjects Two articles are of non clinical origin and concern the placental design as responsible for the gas-exchange between mother and foetus and the foetal glucose metabolism respectively The e papers present excellent reviews of modern concepts on placental and foetal energy exchange and alone justify a purchase of the book

Among the clinical articles in this section one is dealing with the validity of monitoring the human foetal condition during delivery by means of foetal heart rate determination and or acid ba e analysis on foetal scalp blood methods whose place in non academic delivery units still are under dispute Th re is also presented a study on observations performed in cases of iatrogeni foetal hypoxia Two papers on anaesthetic drugs during delivery report pharmacodynamic views and the influence of anaesth ti s on the acid base balance of mother and foetus The section is concluded by a review of the treatment of intrauterine asphyxia and of neonatal hypoglycemia

The second half of the book comprises papers concerned with the use of artificial ventilation of the neon te A technical review of different types of commercially available ventilators precedes a report on the clinical cour e of 119 ventilated children and a pathologist s description of post mortem findings in the lungs of ventilated newborns

Both sections of the course are concluded by a panel discussion

The book offers highly interesting reading It can be strongly recommended to pediatricians and obstetricians because it presents a comprehensive and critical approach to the topics just mentioned. It would be of value for the perinatalogical discipline if this book was also read by physiologists and biochemists inviting them to increased research work in this field

An unsatisfactory proofreading has worried the referee

Gerhard Gennser

R Debre & J Celers (eds) *Clinical virology* Saunders Co Philadelphia London and Toronto 1970 871 pp £16 3s

Th last two decades have witnessed a remarkable and sustained surge of interest in the field of virology The elaboration by J Enders and co-workers, of a method for demonstrating virus in vitro has resulted in the isolation of hundr ds of sorts of viruses from man and other warm blooded vertebrates It has thus been possible to isolate and identify viruses from patients with syndromes of previously only suspected or even unsuspected viral origin This new technique and knowledge have been utilized in clinical practice The results of mass vaccination against poliomyelitis for example in technically dev loped countries is one of th greatest medical successes of the century Several viruses are now on the waiting list for prophylactic vaccination trials

With the leap-frogging progress of today and the immense number of publications scattered among thousands of scientific journals it is extremely difficult, if not impossibl for a single person to grasp the extent of advances in this particular field, to realize the effect of newly acquired knowledg and prophylaxis on the panorama of viral diseases and to compreh nd how elucidation of fundam ntal properties of viruses has facilitat d understanding of previously enigmatic courses of various infe tions

The concise and lucid survey given by Robert Debre and Josette Celers in this book is therefore a welcome newcomer to all workers in this domain

According to th editors, it is a book designed for clinicians It has been written by different authors whose contributions are witn ss to their firm grip of their specialities and their devotion to them

New insights into the nature of viruses and their characteristics naturally lead to grouping of virus species, and in modern textbooks such grouping is

scribed from abroad but, hitherto, no cases have been described in Denmark. The disease is characterized by irregular metaphyses which leave gaping epiphyseal lines frequently accompanied by disproportionate dwarfing and coxa vara.

The vital prognosis is good but dwarfing and bowing of the legs frequently persist. No treatment is possible apart from possible corrective osteotomy. This patient was examined 2 years later. The bowing of the legs had disappeared and the radiographic changes had practically disappeared.

E. W. Flensborg: *Clover-leaf cranium (Kleeblattschädel (Holtermüller-Wiedemann))*

A male infant now aged 4 months with typical symptoms of this syndrome which had been present at birth was demonstrated. There was severe deformity of the cranium to an asymmetrical clover leaf configuration with a large frontal prominence and prominences intero-temporally. The nuchal region was vertically flat with irregular small bony prominences. Pronounced irregular hydrocephalus was present. There was no exophthalmos but stenosis of the lachrymal ducts was present. In addition the patient had bilateral clubfoot deformity, partial syndactyly and congenital morbus cordis and aortic coarctation.

Hitherto 30 cases of clover leaf cranium have been described in the literature. These seem to belong to two different syndromes. The first group have in addition to the clover leaf cranium chondrodystrophy like changes in the bones of the extremities. The second group to which our patient belongs does not show such changes but may have deformities of the extremities of another type particularly clubfoot, anchyloses and syndactyly. Some cases have no deformities apart from those of the cranium. The great majority of cases are German or of German ancestry which also holds true for this patient.

Niels Henrik Valerius: *Focal dermal hypoplasia (Goltz syndrome)*

This patient now aged two years was born at term with a birth weight of 1 800 g. No similar cases were known in the family. An older sister is healthy and the mother had not had any abortions. The diagnosis was established in the neonatal period when the patient was submitted to operation for omphalocele. She has since developed very slowly. At the age of 2 years she weighs 7 200 g and is 75 cm long. She is mentally retarded.

Characteristic skin changes were encountered with areas of up to 2 cm in diameter with atrophy, hyperpigmentation and telangiectases on the trunk and on the face. Microscopic examination from these regions showed changes typical for focal dermal hypoplasia. Small papillomata are present on the inner surface of the lower lip. Syndactyly between the 4th and 5th fingers and toes is present. The nails are dystrophic and are absent on the second finger. Extensive chorioid colobomata are present in both eyes and aniridia in the left eye and coloboma of the iris on the right. The teeth are hypoplastic and not all of them are present. Bilateral harelip and cleft palate are present. The latter malformation has not previously been described in connection with the syndrome. Radiography reveals hypoplasia of the right clavicle. EEG and chromosome investigations reveal normal findings.

Meeting Nov. 10, 1971

Discussion of *Preventive paediatrics and child health in the preschool age*

H. Kreutzfeldt reviewed the present status in Denmark and L. Köhler talked about the Swedish trial with one comprehensive examination of the child 4 years old.

Knud E. Petersen

not only been to describe this syndrome but also in a broader sense the autistic and autistiform behaviour in children The disturbed cognition on the basis of defect perception is well described In the second half of the monograph a case is presented as a new clinical picture in which autistic oligophrenic life is intimately interrelated with a number of innate (genetically determined) bodily malformations This case howev is quite different from those of Kanner s early infantile autism

This publication has the intention to give those who educate multi handicapped children a variegated picture of the autistic syndromes in childhood and their genesis For paediatricians and child psychiatrists there is also much information even if one may have some doubt about the author s wide definition of autistic syndromes The references are comprehensive but the literature is hardly discussed in the book

Lennart Sonesson

D Gairdner & D Hull (eds) *Recent advances in paediatrics* J & A Churchill London 1971 576 pp £6

In the preface to the fourth edition of Recent Advances in Paediatrics" the Editors point out that it is imperative for someone from time to time to put into perspective the mass of new facts and theori s This is certainly a time consuming and diffi ult task which is not becoming easier as years pass The fact that the number of references quoted in the present volume has increased with more than 100 per cent compared with the preceding edition published in 1965 tells its own story The clinician can only feel a d ep gratitude to those experts who are willing to take the great trouble to critically review and in an assimilable form present the results of recent research

The new volume of 'Recent Advances in Paediatrics consi ts of 15 chapters dealing with subjects of current importance and it is highly symptomatic that the first six articles review neonatal problems "The nephrotic syndrome" (illustrated by excellent micro-pho ographs) Immunological mechanisms Immunologi al disorders and Malnutrition in the children of underdeveloped countries" are other examples of chapters of current interest Assessment of endocrine function" reviews in an excellent way ava lable laboratory tests and discusses how and when they should be used.

Space prohibits a more detailed review of all the important contributions to this volume It may however be said that the name of the authors guarant e good reading and for the reviewer it is a pleasure to recommend this new edition of "Recent Advances in Paediatri s

C G Bergstrand

B H Kagan & E. R. Stiehm (eds) *Immuno ogic incompetence* Year Book Medical Publish rs Inc Chicago 1971 US $22 00

The book comprises lectures held at a conference on immunological aspects on the diseases of childhood and includes the subsequent discussions It contains excellent reviews of our present knowledge of the humoral immune system as well as the delayed hyper sensitivity type of immunity Th layout of the presentation is logical and begins with th humoral immunity including the development and structure of the immunoglobulins and the molecular basis of complement activation This is followed by a section on mechanisms of cellular immunity and cellular reactivity including immunologic problems in tumour biology and in pregnancy Clinically important questions are discussed in papers concerning dysfunctions and developmental errors of the synthesis of immunoglobulins Abnormalities of the complement system in relation to disease states and d fects in the effector system of cellular immune reactions are elucidated The relevance of immune mechanisms in the defence against microorganisms is apparent from the analysis of the experiments of nature the immunologic deficiency diseases including syndromes produced by humoral and/or cellular immunologic disorders The role played by phagocytosis in the normal resistance to infections d pends not only on the quality of the phagocytic cells but also on co-factors such as th complement system Diseases referable to disorders in this respect are reported The large body of evidence suggesting involvement of various immunologic disorders in renal diseases is reviewed as well as th aspects on the action of complement and C3 nephritic factor these latter considerations linking th clinical findings closely to recent results suggesting an alternative way of complement fixation The immuno ogic background of allergic diseases is outlined and the possible contributory effect of antibodies of the IgE class is stressed in the provocation of symptoms in atopic allergic diseases

This book is an up-to-date textbook skilfully illustrating the close connection between basic immunology and applied immuno ogy in paediatrics The breakthrou h of immunology in wide and important fields of pa diatrics is obvious Relevant references in th various chapters will be found very useful to those who want to gain a more profound knowledge of the individual subjects

Anna Britta Laurell

J Stoermer & W Heck *Pädiatrische EKG Atlas* (Second Ed) Georg Thiem Verlag Stuttgart 1971 316 pp illus DM 130

This ECG atlas is based on the great experience gained from children studied at the Department of Cardiology of the University Children s Hospital in Göttingen The first edition of th atlas which appeared in 1959 has been revised and considerably expanded. The primary material used for the present edition comprises over 60 000 el ctrocardiograms and

often used as a basis for classification But not in Debre s and Celer s book Division there is by physiological systems and by pathological manifestations and therefore offers the clinician an immediate guide in the investigation and diagnosis of clinical pictures in which viral origin is suspected This is exemplified by the excellent chapters on Viral infections in which neurological manifestations predominate" which is divided into 9 headings discussing diseases of peripheral motor neurons encephalopathies m ningitides and diseases produced by slow viruses etc Similarly the chapters on Viral infections in which cutaneous manifestations predominate and in which respiratory manifestations predominate are divided into 10 and 8 subsections respectively Chapter X Viral infection in relation to other conditions discusses among other subjects prenatal transnatal and post natal viral diseases viruses and chromosomes tumour viruses and vaccination against viral diseases Since a given virus can cause different clinical pictures and ince a given clinical picture may be produced by different viruses a certain degree of repetition is—as pointed out by the authors themselves —unavoidable This minor blemish may however be ignored especially since the book will hardly ever be read from cover to cover at one time The book with its 850 pages is far too comprehensive for a medical student but is recommended to all those who wish to expand their knowledge of virology and to become better acquainted with the way viral diseases appear and behave or to learn how best to utilize the facilities of the virological laboratory for obtaining a decision in suspected cases of diseases of suspected viral origin

Lars Kjellén

V Apgar (ed) *Downs Syndrome (Mongolism)* Ann Acad Sci Vol 171 New York 1970 386 pp US $24 50

This volume contains 41 papers These are the result of a conference entitled Downs Syndrome (Mongolism) held by The New York Academy of Sciences in November 1969 Most of the articles are summary reports and are easily read However the reference information is not the least important part of this volume covering the entire field of mongolism The quality of the articles in such a volume must obviously vary but on the whole they maintain a high standard The first part is devoted to the epidemiology of mongolism but reveals little new In an extensive examination of newborn suspected mongoloids by J Wahrman and K Fried it is noted that no X ray was made of the hand and pelvis skeleton Perhaps the best signs of mongolism are found in the skeleton

The second part deals with chromosomal studies among others cell hybridization and mapping studies in chromosomal abnormalities Unfortunately in this field we are still groping in the dark Caspersson s fluorescence technique which was not evolved until 1970 will probably make a valuable contribution to the solution of these problems

The third part treats environmental factors This section contains several excellent articles The following make interesting reading Australia Antigen Hepatitis Virus and Down s Syndrome by B S Blumberg and co-workers and Thyroid Autoimmunity and Down s Syndrome by P J Fialkow The Physiology of Ovarian Aging is al o discussed in an interesting pap r

In part four there appears a short but clear article on the Premature Senility in Mongolism by G A Jervis A Critical Examination of Some Reported Biochemical Abnormalities in Mongolism by F P Woodford and A G Bearn is also well worth th attention of the reader

In part 5 Clinical Aspects of Mongolism G F Smith describes the various clinical changes at the usual trisomy mosaici m double trisomy and so on J T Queenan reports on Intrauterine Diagnosis of Mongolism In his introductory remarks J W Littlefield expressed the hope that it would gradually be possible to carry out amniocentesis on all pr gnant women aged 40 years or more Neoplasia and Down s Syndrome is discus ed by R W Miller

In the last part Social Considerations for the Retarded Karl Grunewald among oth rs writes about economic opportunities for the mentally retarded Mrs Humphrey wife of the former Vice President of the USA ends the book with a short article whi h in an excellently clear and open manner describes her ideas of how parents of mongoloids experience th ir situation One of her grandchildren is mon oloid She finishes Let us hope that all p ople will soon see their human traits and that there will be a new day in public attitude and receptiven ss toward the mongoloid and toward all who suffer from the affliction of mental retardation

A final appraisal is that the volume has really succeeded in covering the whole fi ld of mongolism including Family Counseling in Down s Syndrome (W R Breg) and that the quality on average is very high All in contact with mongoloids and their families should thus obtain this book

Bertil Hall

J J G Prick *Infantile autistic behaviour and experience* Rotterdam University Press Rotterdam 1971 72 pp D Fl 35 —

This book is the first volume in a serial publication on Modern approaches to the diagnosis and instruction of multi handicapped children It has originated from two papers read at an international conference on the education of deaf blind children at Sint Michielsgestel the Netherlands in 1968 The author Dr J J G Prick is professor of neurology and psychiatry in the Catholic University of Nijmegen

The first half of the book presents general remarks on infantile autistic behaviour and of course there is a detailed description of the characteristic symptoms of Kanner s disease The author s aim however has

and diagnosis where the minor nervous dysfunction is classed in s ven different syndromes The authors say that there is an overlap b twe n th groups The diagnosis of minor nervous dysfunction is s ttled when evidence of a clear cut neuro ogical disease such as hemipl gia or ataxia do s not exist There are minor abnormalities giving the picture of the clumsy chi d often with a borderline or definite psychiatric diagnosis

The book is recommended for tho e who try to understand and evaluate children with thes minor abnormalities

Ingrid Bjerre

A B B rgman J B Beckwith & C G Ray (eds) *Sudden infa it death syndrome* University of Washington Press Seattle 1970 295 pp illus US $10 00 £4 75

This very well edited fascinating volum brings together recent epidemiologic morpholo ic virologic and physiolo ic studies on the sudden infant death syndrome (SIDS) The entity (?) formerly referred to as sudd n unexpected death or crib death obviously constitutes an important medical problem perhaps not fully appreciated by physicians outside the departments of forensic medicine The incidence of sudden infant death in the western countri s thus ranges between 1 and 3 0 per 1 000 live births The disease affects characteristically infants below the age of 6 months and particularly babies from lower socio- conomic conditions There is also a pre dominance of SIDS among infants born prematurely

Various theories on the pathogenesis of sudden in fant death have been presented throughout th years but the basic underlying m chani ms remain unex plained There is no evidence of any specific laryng al tracheo-bronchial or pulmonary lesions in these cases althou h many victims display some degree of inflammatory changes in the respiratory tract in addi tion to the frequ ntly occurring intrathoracic pe techiae In the book no reference is made to the possibility that sudden d ath in infancy could be the manifestation of silent pu monary hypertension It might be added to the patholo y discussion that a careful histologic study of th pulmonary vasculature the muscular pulmonary arteries in particular should be carried out in each case of SIDS

In 1967 1968 a eries of publications by Dr P Geertinger suggested that parathyroid insuffici ncy was the h therto overlooked cause of sudden infant death This amazin concept was based upon mor pho ogic observations i e ab ence of parathyroid tissue or fusion of parathyroid and thymic tissue in larger proportion of SIDS cas s than in controls At this meeting the th ories of Dr Geertinger was in his absence strongly challenged by Dr Va des Dapena who reported that she by means of microdissection and sub equent histolo ic examination had been able to demonstrate two or more normal parathyroids in 96 of SIDS cases and in 86 of contro s Thus there seems to be no morpholo i reason for in criminating parathyroid insufficiency among the pos ible etio ogic factors in SIDS

Th virologic aspects of the prob em have b en somewhat more r warding than th morpho ogic approach A definite increase of positive virus cultures from various organ samples has been reported in SIDS victims but o far th si ificanc of this finding is uncertain Perhaps the mo t adequate char acterization of this book is provid d by th sum mary statement of Dr J Houstek Pragu I still have th feeling that in spite of th various theories mentioned at this conference th answer may lie elsewh re"

Bengt Robert on

U H Krech M Jun & F Jung *Cytomega ovirus infections of man* S Karger AG Ba el 1971 124 pp DM 32 —

In recent years increasing attention has been paid to cytomegalovirus (CMV) infections in man Previously confirmation of the diagnosis was based on pathological or cyto ogical findings but the i olation of human CMV achieved in 1956 made cultural and erolo ical m thods possib e In mo t inst nces these techniqu s are preferable CMV has been proved to give ri e to as varying symptoms as P ul Bunnell negative mononucleosis hepato plenomegaly post perfusion syndrome and cong nital malformations The most serious complication of congenital infection is mental retardation The authors have taken special interest in the problems of congenital and neonatal in fections which can be difficult to separat In a few pages th book provides excellent information on practical problems concerning virolo y epidemiology pathogene i clinic and labora ory diagnosi of human CMV infections It contains 350 references and tables giving a good survey of different investigations The results are partly based on th authors own investi a tions The book is of practical value for clinicians as well as for laboratory do tors

Ka in Stormby

large clinical series including 10 000 children with congenital heart disease

The general part of the atlas (36 pages) deals with the following main topics Special features of the electrocardiogram in infants and children normal values positions of the heart cardiac hypertrophy vector cardiography and intracardiac electrocardiography Normal values were derived from 1 000 electrocardiograms registered in healthy newborns infants and children divided into 8 age groups Mean values and normal ranges of variation are presented for each group in easily comprehensible and useful tables

The atlas proper comprises a large collection of electrocardiograms with interpretations First examples are given of normal tracings artifacts ar rhythmias conduction disturbances and orthostatic changes Electrocardiograms of children with congenital heart disease form the major part of the atlas Practically all cardiac malformations are represented Examples are also given of postoperative changes in the electrogram Furthermore the atlas illustrates electrocardiographic changes in carditis acquired heart lesions systemic and pulmonary hypertension metabolic disorders electrolyte disturbances etc A great merit of the atlas is that the electrocardiograms are supplemented by clinical data on the patients X ray findings and results of cardiac catheterization and angiocardiography Post mortem findings are presented in fatal cases Hence the electrocardiograms are interpreted in the light of the prevailing clinical hemodynamic and anatomical conditions The atlas comprises 256 figures of a high technical quality 22 tables and 500 references It has strengthened its position as a standard book of reference in paediatric cardiology

B Landtman

M Ingle Wright *The pathology of deafness An introduction* Manchester University Press The Williams & Wilkins Co Manchester and Baltimore 1971 178 pp £2 64

The author Senior lecturer in otolaryngology University of Manchester has written a short but comprehensive book on the causes of deafness

The usual clinical distinction between conductive and perceptive deafness is not made The classification is based on pathology and includes chapters on congenital malformations maternal diseases during pregnancy hormonal defects and ototoxic agents Also deafness in association with familial dis a e hereditary disorders of connective tissue and collagen diseases mucopolysaccharidoses di orders of nutrition and reticulo-endothelial system Much space is devoted to both pathogens and d fences in infection

This book is well worth reading especially recommended to paediatricians since many—but rare—complex diseases of childhood involve deafness or impaired hearing

Peter Groth

E Schenk *Neurologische Untersuchungsmethoden* Georg Thieme Verlag Stuttgart 1971 252 pp illus. DM 13 80

This handbook written in German and of pocket size is very comprehensive The different neurological examinations are grouped in chapters in a practical way for clinical use There are also short comments about psychological methods and descriptions of the different laboratory methods in neurology

I think the book will be of practical use in daily work as a support for the memory

Ingrid Bjerre

E Gautier & L S Prod hom (eds) *Hämatologie im Kindesalter* Pädiatrische Fortbildungskurse für die Praxis Vol 31 S Karger Basel 1971 125 pp illus. sFr 35 - US $8 00

Hämatologie im Kindesalter is a new volume in the series Pädiatrische Fortbildungskurse für die Praxis" Wellknown European paediatricians give reviews of current interest on eight subjects Within the field of erythropoiesis there are two papers Betke presents foetal and postnatal erythropoiesis and its disturbances and Tonz reviews hereditary disorders of the red blood cells G de Muralt discusses prevention of Rh iso-immunization Two papers concern haemostasis one by Kunzer dealing with coagulation in the newborn and its disturbances the other by Alagille regarding neonatal thrombocytopenias Another two papers concern the leucocytes Jeannet writes about the HL A system and Hitzig about disturbances in the function of the granulocytes Finally Bernard summarizes the recent pro ress in the treatment of acute leukaemias in childhood In all these fields our knowledge has increased consid rably during the last few years not the least thanks to important contributions from the pres nt authors Their summing ups are clear and concise with excellent schematic figures and tables The volume can be recommended to all paediatricians interest d in haematology

Hans Ekelund

B Touwen & H Prechtl *The neurological examination of the child with minor nervous dysfunction* Clinics in Developmental Medicine no 38 William Heinemann Medical Books Ltd London 1970 105 pp illus £2 25

A very difficult part of pediatric neurology the minor nervous dysfunction is discussed in this book The authors give an extensive account of the approach to these children with the aid of a neurological examination which in many ways differs from the conventional The most valuable part of the book is the thorough description of how to make this examination and the evaluation of the results The clinical methods are illustrated by very instructive pictures

The detailed form used to register the findings will certainly not be used by many pediatricians I am also a little doubtful about the chapter interpretation

Acta Pædiat Scand 61 517-525 1972

HAEMOPHILIA A AND VON WILLEBRAND S DISEASE IN A SWEDISH FAMILY

L HOLMBERG and I M NILSSON

From the Coagulation Laboratory and the Department of Paediatrics Malmo Allmanna Sjukhus Malmo Sweden

Haemophilia A and von Willebrand s disease are two ha morrhagic diatheses whose clinical and laboratory characteristics are now fairly well known (13 14 15 23 25) The prevalence of haemophilia A in Sweden is 1 per 15 000 males and that of von Willebrand s disease 1 per 16 000 inhabitants (25) The genes for these diseases are inherited independently of one another the gene for haemophilia A being X-chromosomal and recessive and that for von Willebrand s disease being autosomal and dominant

The probability of both diseases occurring in one and the same family is small and would still be so even if von Willebrand s disease w re more common than widely supposed A population of at least 40 million people with the same frequency of the genes as in Sweden would be required before one could expect to encounter *one* family with both diseases

Both haemophilia A and von Willebrand s disease are characterised by a reduced activity of AHF which is thus under the control of two different genetic loci In von Willebrand s disease also the bleeding time is prolonged be cause of deficiency of a plasma factor oc curring in normal plasma and in haemophilia A plasma and recovered in fraction I-O (13) von Willebrand factor is not identical with AHF It normalises the prolonged bleeding time in von Willebrand s disease and infusion of fraction I O in patients with this disease is followed by a characteristic progressive in crease of AHF Such an increase may be due to a true synthesis of AHF or possibly to activation of an inactive molecule

On the basis of findings in experiments with rabbit antiserum against AHF it has recently been claimed that haemophilia A plasma con tains a protein immunologically related to AHF (2) but that this protein is missing in von Willebrand s disease (26 27) Most pa tients with haemophilia A however lack the ability to neutralise a human inhibitor of AHF (6 9)

This paper reports a family with both haemophilia A and von Willebrand s disease A similar American family has been published by Geiger & Rath (7) The relationships be tween the members have been confirmed sero logically The family has been traced back to the 18th century We have had the opportunity of examining most of the members still living Conventional diagnostic methods were used We have also examined the plasma for AHF related antigenic material with a human in hibitor of AHF as well as with a specific rabbit antiserum

MATERIAL AND METHODS

AHF-con entrate

The AHF-concentrate used was fraction I O prepared by AB KABI (AHF KABI) according to Blomback & Blomback (4)

ANNOUNCEMENTS

CLINICAL NEUROLOGY INFORMATION CENTER

In March 1972 the Clinical Neurology Information Center (CNIC) was established at the University of Nebraska College of Medicine under the auspices of the National Institute of Neurological Diseases and Stroke This is the third of a series of information centers in the NINDS Neurological Information Network Brain Information Service is at UCLA and the Information Center for Hearing Speech and Disorders of Human Communication is at Johns Hopkins

The initial activities of CNIC will be the publication of State of the Art papers these will be critical reviews of topics of interest to neurologists neurosurgeons and other clinical neuroscientists

Information concerning CNIC may be obtained by addressing inquiries to Director Clinical Neurology Information Center Medical Library University of Nebraska College of Medicine Omaha Nebraska USA 68105

SECOND ANNUAL MEETING OF THE ASSOCIATION FOR PEDIATRIC EDUCATION IN EUROPE

The second annual meeting of the Association for Pediatric Education in Europe will take place in Hellebæk near Copenhagen from the evening of Friday September 29th till noon Sunday October 1st immediately following the 4th World Conference on Medical Education For information write to the Secretary of A P E E Professor Spyro Doxiadis, Institute of Child Health Athens 608 Greece

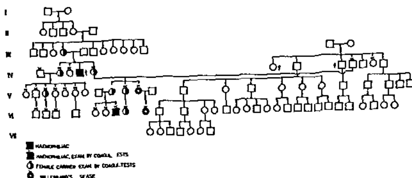


Fig 1 Pedigree of family with haemophilia A and von Willebrand's disease

IV 9 E A retired carpenter No bleeding symptoms On one occasion he had cut off the middle finger of his left hand and on another he had injured his right leg with an axe without any serious complications on either occasion He wears a full denture

IV 10 F A died from tuberculosis

IV 11 E T married no children Operated upon because of extra uterine pregnancy Menopause previously normal menstruation Abnormal tendency to haematoma after intramuscular injections Ready bruisability No abnormal bleeding after tooth extraction When young she often had anaemia

V 3 B P had suspect renal bleeding in association with delivery and was given a plasma transfusion Otherwise no abnormal bleeding except nose bleeding

V 9 I H no abnormal bleedings Does not use oral contraceptives

V 10 G A had often had gingival bleedings as a child Her blood values were always poor Heavy bleeding after parturition but menstruations normal. Bruised readily

V 11 A G A menstruation normal Bled somewhat post partum No manifestations of bleeding with certainty Uses oral contraceptives

VI 7 B H born 1964 At 16 months the patient had severe bleeding from the upper lip He became anaemic and was given blood transfusions From 2 / years of age he had joint bleedings every month particularly in the right knee but also in the right elbow and left ankle He has a slight extension defect outward rotation and valgus deformity of the right knee He has also had muscle bleedings and bleeding from the oral cavity He can manage without orthopaedic appliances but can only walk short distances out of doors

VI 9 A L A born 1966 Had a spell of petechiae in the skin at 9 months of age soon afterwards also haematoma in the skin At 11 months of age she had haematuria and the Hb fell to 4 0 g/100 ml From 1 year of age onwards she had cutaneous bleedings and muscle bleedings as well as haemarthrosis of the ankles knees and shoulders She also had haemorrhage from the floor of the mouth and from the gingiva These bleeding episodes occurred at short intervals and she was admitted to hospital on 28 occasions in the course of 4 years

LABORATORY STUDIES

The results of blood grouping are given in Tables I *a* and I *b* The examination comprised the usual blood groups including the sex linked group Xg Determinations were also made of the genetic variations of several serum proteins and of red cell enzymes

A complete examination of the coagulation status was made in V 11 and V 12 and VI 7 and VI 9 The results are given in Table 2 In the other members determinations were made of at least AHF and bleeding time (Table 3)

IV 2 had a normal AHF (menopause) and normal bleeding time

IV 3 was normal

IV 5 had a normal AHF but she too is in the menopause Her bleeding time was normal

IV 9 was normal

IV 11 had a normal content of AHF and normal bleeding time Salzman 17–24 No progressive AHF increase occurred after infusion of 300 ml AHF KABI

V 3 had a reduced amount of AHF The bleeding time was normal Infusion of 200 ml AHF KABI raised the AHF level proportional to the amount of AHF infused (Fig 2 *a*) AHF from the same batch was given to a patient with haemophilia A and a corresponding increase was noted Neutralisation of the human inhibitor was found (Table 4)

Methods

The methods used for determining the various coagulation factors and components of the fibrinolytic system have been described previously The investigation comprised determination of the bleeding time according to Duke and to Ivy (5 18) coagulation time recalcification time prothrombin consumption test factor V factor VIII factor IX factors XI and XII one stage prothrombin time P & P fibrinogen and components of the fibrinolytic system (12 14 16 17) The platelet count was determined by the method of Bjorkman (3) and platelet adhesiveness according to Hellem (8) and Salzman (24)

Quantitative determination of inhibitor of factor VIII

The plasma to be tested (0 6 ml) was incubated in various dilutions (1/1 1/2 1 5 and 1/10) with human AHF concentrate (0 2 ml) (fraction I O) at 37 C for 2 hours As a blank 0 6 ml saline was incubated with 0 2 ml of the AHF concentrate Following incubation the blank and the mixtures of plasma and AHF concentrate were assayed for residual factor VIII activity according to the method of Nilsson Blomback & von Francken (12) The inhibitory activity of the plasma was expressed as the number of units of factor VIII (one unit of factor VIII is defined as the amount of factor VIII present in 1 ml of normal plasma) inactivated by 1 ml of the plasma

Ability of plasma to neutralise an inhibitor of AHF

This was assayed according to a modification of Dinsons method (6) Plasma from a patient with severe haemophilia A who had developed an anticoagulant against AHF was used as a source of inhibitor The inhibitory activity of the plasma was about 3 units of factor VIII per ml

The test was performed in the following way 0 1 ml of the Al(OH) adsorbed inhibitory plasma was incubated with 0 5 ml of the plasma to be tested and with blank respectively for 4 hours at 37 C (step 1) The blank consisted of one part of 3 8 sodium citrate and 5 4 parts of NaCl Normal plasma (pool from ten registered blood donors) was always incubated in the same way as a control

After incubation the samples were stored in refrigerator at +4 C over night The following morning residual inhibitor was quantitatively determined with the method described above (step 2) As pointed out one inhibitor unit (IU) was defined as the amount of inhibitor that neutralised one unit (U) of AHF

The ability of the plasma to neutralise the inhibitor was calculated in the following way

x residual amount of AHF in U/ml in step 2 after incubation with plasma (normal or test) in step 1
y residual AHF in U/ml in step 2 after incubation with blank in step 1
a ml added AHF concentrate in step 2
b ml blank and plasma respectively in step 1
c ml adsorbed inhibitory plasma in step 1

If k is the activity in the AHF concentrate the mixture of plasma and inhibitor will have neutralised $ka - x(a+b+c)$ and the mixture of blank and inhibitor $ka - y(a+b+c)$ units of AHF The respective mixtures thus contained by definition corresponding amounts of residual inhibitor in IU after the incubation in step 1 The difference in the amount of residual inhibitor between the blank and the plasma mixtures will be a measure of the amount of inhibitor neutralised by plasma

$$ka - y(a+b+c) - ka + x(a+b+c) = (x-y)(a+b+c)$$

IU or per ml plasma

$$\frac{(x-y)(a+b+c)}{b} \text{ IU/ml}$$

Optimal proportions of a b and c are those which give a maximum for this fraction A prerequisite is that $x < 0.25k$ i e that the inhibitor is added in excess

Preparation of rabbit antiserum

AHF was purified in the following way Fraction I O (AHF KABI) was used as starting material A separation procedure according to v Mourik & Mochtar (11) was used 15 ml newly dissolved fraction I O was filtered on a column with Sepharose 6 B using an eluting buffer containing Rheomacrodex We obtained an almost homogenous preparation with the void volume containing an AHF activity of 100 (1 unit per ml) after concentration This preparation was used for immunising rabbits The antiserum had a strong inhibitory activity against AHF and gave one precipitation line on agarose immune electrophoresis (21) (An initial batch of antiserum for preliminary experiments and for comparison was kindly supplied by Dr Bouma Utrecht the Netherlands)

Immunologic determination of AHF

AHF was determined immunologically with the technique of Laurell (10) i e electrophoresis in agarose gel containing antibodies The buffer used did not contain calcium The determination was made on cryoprecipitate of plasma

3 ml was frozen to −60 C for 1 hour and thawed at +6 C for 8 hours after which the plasma was centrifuged at +4 C and at 4 000 *g* for 15 minutes The precipitate was dissolved in 0 5 ml of 0 075 M diemalbuffer pH 8 6 at 37 C

Clinical material

The pedigree is given in Fig 1

IV 4 E A died in 1918 at the age of 1 years Spent many months in hospital probably because of intracranial haemorrhage Cause of death bleeding from a wound in the chin

IV 5 A A bled profusely after operation for uterine prolapse but otherwise no abnormal bleeding

IV 6 A A in advanced age admitted to hospital because of severe anaemia which was thought to be pernicious on one occasion but sideropenic on another Nose bleedings

IV 8 committed suicide

to 3 minutes The AHF activity rose imme diately to 67 / (Fig 2*c*) Owing to sampling difficulties it was not possible to follow the immediate course of AHF activity Also VI 9 had no ability to neutralise the inhibitor of AHF Immunologically AHF was 64°

DISCUSSION

Nothing in the serological examination sug gested that the family relationships reported were not correct The family thus comprised two cousins a boy and a girl with severe bleeding symptoms The boy VI 7 has had joint bleedings since the age of 2¹/ years and muscle bleedings His right knee showed arthropathy grade 3 (1) He was somewhat disabled The symptoms are typical of haemophilia In severe von Willebrand s disease dis ability rarely if ever occurs despite impaired

Table 2 *Coagulation status of four members of family VI 7 with haemophilia A VI 9 and V 11 with von Willebrand s disease and V 12 unaffect ed father of VI 9*

	V 11	VI 9	VI 7	V 12
Coagulation time				
glass tube (min)	18	21	[illegible]2	9
plastic tube (min)	[illegible]5	>60	36–50	16
Bleeding time				
Duke (min)	3	30	2	3
Ivy (min)	21	>30	14	7
Platelets per mm	204 000	30[illegible] 000		218 000
Platelet adhesiveness				
Hellem ( )	20	16		
Salzman ( )	21	18	[illegible]0	21
Prothrombin consumption ( )	11	46		22
AHF (factor VIII) ( )	57	5	2	115
Factor IX (%)	90	75	64	117
Factor IX factor XII ( )	117	98		168
Owren s P&P ( )	9[illegible]	112	108	88
Factor V ( )	90	98	137	95
Fibrinogen (g/100 ml)	0 51	0 31	0 44	0 34
Fibrinolytic activity on unheated plates				
plasma (mm²)	0	45		0
resusp euglob prec (mm)	70	1[illegible]0		0
Euglobulin clot lysis time (min)	>1[illegible]0	60		>1[illegible]0
FDP (mg/100 ml)	0	0		0
Anticoagulant		0	0	

Table 3 *AHF and bleeding time in members of family*

	AHF ( )	Bleeding time (min)	
		Duke	Ivy
IV 1	138	2	10
IV 2	82	2	14
	80		
IV 3	225	1	6
	285		
IV 5	132	2–5	10
IV 9	102	2	9
IV 11	76–100	2	12
V 1	67–128	2	11
V 2	78	1	6
V 3	48	5	13
V 4	104	2	10
V 5	123	3	9
V 6	112	2	14
V 9	50–64		
V 10	46–50	2	8
			10
VI 1	70	1	7
VI 2	115	2	
VI 3	75		
VI 5	90		11
VI 6	83		
VI 8	45–48	1	7

mobility of single joints (25) Analysis of the coagulation system also showed that the pa tient has haemophilia A of moderate severity No evidence of von Willebrand s disease was found the bleeding time by the method of Duke and of Ivy as well as platelet adhesive ness according to Salzman were normal His response to infusion of AHF was also typical of haemophilia A

The symptoms in the female cousin VI 9 appeared already before 1 year of age with *cutaneous bleedings and haematuria She then* had not only gingival bleeding and cutaneous bleeding but also joint bleedings Though these bleedings had been frequent they had not resulted in any permanent impairment of function or of disability Her coagulation status was typical of severe von Willebrand s disease with an AHF of 5 and markedly prolonged bleeding time Infusion of AHF produced complete normalisation of the bleeding time which is so characteristic of von Willebrand s disease but the progressive increase of AHF could not be demonstrated owing to sampling

Table 1 *a Blood groups*

	ABO	Rh	MNS	P	K	Fy(a)	Fy(b)	Jk(a)	Jk(b)	Xg(a)
IV 1	A_2	—	MNss	−	−	+	+	+	+	+
IV 2	O	—	MNSs	+	−	+	+	+	+	+
IV 3	B	—	Nss	+	−	−	−	+	+	
IV 5	B	—	Nss	+	−	−		+	−	+
IV 9	O	Rh_2rh	Nss	+	−	+	+	−		−
V 2	O	—	MSs	−	−	+	+	+	−	−
V 3	A_2	—	MSs		−	+	−	+	+	
V 5	O	—	MNSs	−	−	+	+	+	+	
V 6	O	—	MNSs	+	−	+	−	+	+	
V 8	A_1	Rh_2rh	MNss	+	−	+	+	−		+
V 9	O	—	Nss	+	−	+	+	+	+	+
V 10	B	—	Nss	+	−	−		+	+	+
V 11	O	—	Nss	+	−	+	+	+	+	
VI 1	O	Rh_1rh	MSs	+	−	+	+	+	−	+
VI 5	A_1	—	Nss	+	−	+	+	+	+	+
VI 6	O	—	Nss	+	−	−		+	+	+
VI 7	A_1	—	MNss	+	−	−		−		−
VI 8	A_1	Rh_1rh	MNSs	+	−	+	+	+	−	−
VI 9	O	Rh_1rh	Nss	+	−	−		−	+	

V 9 Decreased content of AHF in one sample and normal in another Ability to neutralise the inhibitor was low The amount of immuno logically demonstrable AHF as shown with rabbit antiserum was 100% (Table 5)

V 10 had a low AHF content and normal bleeding time according to the method of Ivy Platelet adhesiveness according to Salzman was 21% Infusion of 300 ml AHF KABI produced an increase in the AHF activity corresponding to that in vitro (Fig 2 *a*)

Table 1 *b Serum types and red cell enzymes*

	Gm(l)	Gc	Hp	Ag(x)	PGM	EAP	AK
IV 1	+	2-1	1-1	−	2 1	AA	1-1
IV 2	−	1-1	2-2		2-1	BA	1-1
IV 3	−	1-1	2-2	−	1 1	BA	1-1
IV 5	−	1-1	2-2		2-1	AA	1-1
IV 9	+	1-1	2-1	+	1 1	BA	1-1
V 2	+	1-1	2-1		2-2	AA	1-1
V 3	+	1-1	2-1	−	2-1	AA	1-1
V 5	+	1-1	2-1	−	2 1	AA	1-1
V 6	+	1-1	2-1		1-1	BA	1-1
V 8	+	2-1	2-1	−	1-1	CA	1-1
V 9	+	1-1	2-2	+	2-1	AA	1-1
V 10	−	1-1	2-2	+	2 1	AA	1-1
V 11	+	1-1	2-1	+	2-1	BA	1-1
VI 1	−	1-1	2-2	−	2 2	AA	1-1
VI 5	+	2-1	2-1	−	2-1	AA	1-1
VI 6	+	2-1	2-2	−	2-1	CA	1-1
VI 7	+	1-1		+	2-1	CA	1-1
VI 8	−	1-1	2-1	+	1-1	BA	1-1
VI 9		1-1	1-1	+	1-1	CA	1-1

Ability to neutralise the inhibitor existed but was fairly small in the two tests performed

V 11 had a prolonged Ivy bleeding time and an AHF content of 57% while using oral contraceptives Platelet adhesiveness accord ing to Salzman 21% Plasma from V 11 neu tralised an inhibitor of AHF to the same extent as plasma from healthy members of the family and as normal plasma Immuno-logically determined AHF was 92%

V 12 was normal

VI 7 had an AHF content of 2–4% but a normal bleeding time according to Duke and according to Ivy Platelet adhesiveness accord ing to Salzman 20% He was given 100 ml AHF KABI The AHF activity increased from 4 to 25% and then fell rapidly (Fig 2 *b*) He could not neutralise an inhibitor of AHF Immunologically AHF was 145%

VI 8 had a reduced AHF content and normal bleeding time

VI 9 is a daughter of V 11 and V 12 Her bleeding time according to the methods of Duke and of Ivy was markedly prolonged AHF 5% The patient was given 200 ml AHF KABI This normalised the bleeding time ac cording to Duke which fell from 30 minutes

to 3 minutes The AHF activity rose immediately to 67% (Fig 2 *c*) Owing to sampling difficulties it was not possible to follow the immediate course of AHF activity Also VI 9 had no ability to neutralise the inhibitor of AHF Immunologically AHF was 64%

DISCUSSION

Nothing in the serological examination suggested that the family relationships reported were not correct The family thus comprised two cousins a boy and a girl with severe bleeding symptoms The boy VI 7 has had joint bleedings since the age of 2¹/ years and muscle bleedings His right knee showed arthropathy grade 3 (1) He was somewhat disabled The symptoms are typical of haemophilia In severe von Willebrand s disease disability rarely if ever occurs despite impaired

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Factor IX ( )	90	75	64	117
Factor IX + factor XII ( )	117	98		168
Owren s P&P ( )	92	112	108	88
Factor V (%)	90	98	137	95
Fibrinogen (g/100 ml)	0 51	0 31	0 44	0 34
Fibrinolytic activity on unheated plates				
plasma (mm)	0	45		0
resusp euglob prec (mm)	70	120		0
Euglobulin clot lysis time (min)	>1?0	60		>120
FDP (mg/100 ml)	0	0		0
Anticoagulant		0	0	

Table 3 *AHF and bleeding time in members of family*

	AHF (%)	Bleeding time (min)	
		Duke	Ivy
IV 1	138	2	10
IV 2	82	2	14
	80		
IV 3	2.5	1	6
	285		
IV 5	132	2 5	10
IV 9	102	2	9
IV 11	76–100	2	12
V 1	67–1?8	2	11
V 2	78	1	6
V 3	48	5	13
V 4	104	2	10
V 5	123	3	9
V 6	112	2	14
V 9	50–64		
V 10	46–50	2	8
			10
VI 1	70	1	7
VI 2	115	2	
VI 3	75		
VI 5	90		11
VI 6	83		
VI 8	45–48	1	7

mobility of single joints (25) Analysis of the coagulation system also showed that the patient has haemophilia A of moderate severity No evidence of von Willebrand s disease was found the bleeding time by the method of Duke and of Ivy as well as platelet adhesiveness according to Salzman were normal His response to infusion of AHF was also typical of haemophilia A

The symptoms in the female cousin VI 9 appeared already before 1 year of age with cutaneous bleedings and haematuria She then had not only gingival bleeding and cutaneous bleeding but also joint bleedings Though these bleedings had been frequent they had not resulted in any permanent impairment of function or of disability Her coagulation status was typical of severe von Willebrand s disease with an AHF of 5 and markedly prolonged bleeding time Infusion of AHF produced complete normalisation of the bleeding time which is so characteristic of von Willebrand s disease but the progressive increase of AHF could not be demonstrated owing to sampling

Table 1*a* *Blood groups*

	ABO	Rh	MNS	P	K	Fy(a)	Fy(b)	Jk(a)	Jk(b)	Le(a)
IV 1	A_2	—	MNss	–	–	+	+	+	–	+
IV 2	O	—	MNSs	+	–	+	+	+	+	–
IV 3	B	—	Nss	+	–	–	–	+	+	
IV 5	B	—	Nss	+	–	–		+	–	+
IV 9	O	Rh_1rh	Nss	+	–	+	+	–		–
V 2	O	—	MSs	–	–	+	+	+	–	–
V 3	A_2	—	MSs		–	+	–	+	+	
V 5	O	—	MNSs	–	–	+	+	+	+	
V 6	O	—	MNSs	+	–	+	–	+	+	
V 8	A_1	Rh_1rh	MNss		–	+	+	–		+
V 9	O	—	Nss	+	–	+	+	–	+	+
V 10	B	—	Nss	+	–	–		+	–	+
V 11	O	—	Nss	+	–	+	+	+	+	
VI 1	O	Rh_1rh	MSs	+	–	+	+	+	–	+
VI 5	A_1	—	Nss	+	–	+	+	+	+	+
VI 6	O	—	Nss	+	–	–		+	+	+
VI 7	A_1	—	MNss	–	–	–		–		–
VI 8	A_1	Rh_1rh	MNSs	+	–	–	+	+	–	–
VI 9	O	Rh_1rh	Nss	+	–			–	+	

V 9 Decreased content of AHF in one sample and normal in another Ability to neutralise the inhibitor was low The amount of immuno logically demonstrable AHF as shown with rabbit antiserum was 100% (Table 5)

V 10 had a low AHF-content and normal bleeding time according to the method of Ivy Platelet adhesiveness according to Salzman was 21% Infusion of 300 ml AHF KABI produced an increase in the AHF activity corresponding to that in vitro (Fig 2*a*)

Table 1*b* *Serum types and red cell enzymes*

	Gm(l)	Gc	Hp	Ag(x)	PGM	EAP	AK
IV 1	+	2-1	1-1	–	2-1	AA	1-1
IV 2	–	1-1	2-2	–	2-1	BA	1-1
IV 3	–	1-1	2-2		1-1	BA	1-1
IV 5	–	1-1	2-2	–	2-1	AA	1-1
IV 9	+	1-1	2-1		1-1	BA	1-1
V 2	+	1-1	2-1	–	2-2	AA	1-1
V 3	+	1-1	2-1	–	2-1	AA	1-1
V 5	+	1-1	2-1	–	2 1	AA	1-1
V 6	+	1-1	2-1	–	1-1	BA	1-1
V 8	+	2-1	2-1	–	1-1	CA	1-1
V 9	+	1-1	2-2	+	2-1	AA	1-1
V 10	–	1-1	2-2	+	2-1	AA	1-1
V 11	+	1-1	2-1	+	2-1	BA	1-1
VI 1	–	1-1	2-2	–	2-2	AA	1-1
VI 5	+	2-1	2-1	–	2-1	AA	1-1
VI 6	+	2-1	2-2	–	2-1	CA	1-1
VI 7	+	1-1		+	2-1	CA	1-1
VI 8	–	1-1	2-1	+	1-1	BA	1-1
VI 9		1-1	1-1	+	1-1	CA	1-1

Ability to neutralise the inhibitor existed but was fairly small in the two tests performed

V 11 had a prolonged Ivy bleeding time and an AHF content of 57% while using oral contraceptives Platelet adhesiveness according to Salzman 21% Plasma from V 11 neutralised an inhibitor of AHF to the same extent as plasma from healthy members of the family and as normal plasma Immunologically determined AHF was 92%

V 12 was normal

VI 7 had an AHF content of 2–4% but a normal bleeding time according to Duke and according to Ivy Platelet adhesiveness according to Salzman 20% He was given 100 ml AHF KABI The AHF activity increased from 4 to 25% and then fell rapidly (Fig 2*b*) He could not neutralise an inhibitor of AHF Immunologically AHF was 145%

VI 8 had a reduced AHF content and normal bleeding time

VI 9 is a daughter of V 11 and V 12 Her bleeding time according to the methods of Duke and of Ivy was markedly prolonged AHF 5% The patient was given 200 ml AHF KABI This normalised the bleeding time according to Duke, which fell from 30 minutes

tory findings are compatible with her being a carrier

All the living potential carriers except V 7 have been examined IV 2 had a normal AHF but is in the menopause V 3 and V 10 in generation V had low AHF values on repeated occasions Both had normal bleeding times After infusion of AHF both had a rise of the AHF activity corresponding to the activity of the infused preparation *in vitro*

Thus nothing suggests that V 3 and V 10 have von Willebrand s disease both are carriers of haemophilia A VI 8 who is daughter of V 10 and who has not had any bleeding symptoms is probably also a carrier of haemophilia A

The father V 12 of the girl with severe von Willebrand s disease had a normal coagulation status and he has never had any bleeding symptoms The mother V 11 has never had any bleeding symptoms either But her AHF during the use of oral contraceptives was 57 which must be considered to be low (20) Her bleeding time according to Ivy was also clearly prolonged These changes are compatible with mild von Willebrand s disease

There are no further cases of von Willebrand s disease among the offspring of III 4 a d III 5 Neither are there any cases of the disease among offspring of III 12 and III 13 IV 9 has no bleeding history and his coagulation status is normal There is no reason to suppose that IV 6 had the disease but her coagulation status was never examined before death IV 11 has it is true had bleeding symptoms but investigation excluded the possibility of von Willebrand s disease Thorough investigation of relatives of III 12 and III 13 revealed no signs of abnormal bleeding in any of them

von Willebrand s disease thus occurs first in the family with V 11 Owing to the incomplete penetrance (25) it is however not possible to demonstrate with statistical significance that it is a new mutation

Using an immunological method an AHF related protein was found in high concentration in the patient with haemophilia A and in normal concentration in his mother who is a carrier Both of the patients with von Willebrand s disease had a normal content of AHF immunologically in contrast with most of the patients with this disease (22)

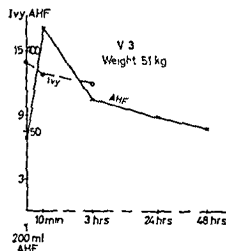
Nevertheless VI 7 and VI 9 i e the patients with moderate haemophilia A and severe von Willebrand s disease could not neutralise a human inhibitor of AHF while V 11 with mild von Willebrand s disease could The carriers of haemophilia A could also neutralise the inhibitor and could not be clearly distinguished as a group from healthy relatives and normals V 3 V 9 and V 10 however had low values in an intermediate zone between those of VI 7 and VI 9 on the one hand and those of normal persons on the other

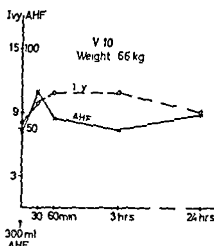
Much suggests that both AHF and von Willebrand factor activities reside in one and the same high molecular weight protein complex It is this complex that is demonstrated by the immunological method (22) In the patient with moderate haemophilia A the AHF part of the complex may be missing or malformed but the von Willebrand factor is still immunologically demonstrable If the AHF is malformed the malformation must affect that part of the molecule against which the human inhibitor is directed because it is not neutralised by the patient s AHF

The carriers of haemophilia presumably have two populations of AHF one normal and one malformed This assumption is supported by the decreased ability of three of the carriers to neutralise the inhibitor

We have recently examined twelve families with von Willebrand s disease with the immunological method In ten of the families the affected members had a low content of the AHF von Willebrand factor protein but in the remaining two the content was normal (22) This suggests the existence of a genetic variant of von Willebrand s disease

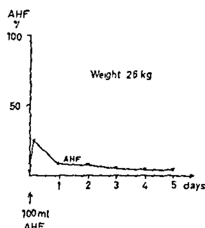
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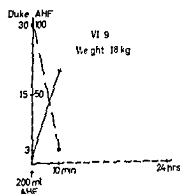


a

Fig 2 AHF activity and bleeding time after infusion of fraction I O in (*a*) two carriers of haemophilia A (*b*) patient with haemophilia A (*c*) patient with von Willebrand s disease



b



c

difficulties The bleeding time remained normal for at least one day It is not possible to decide whether VI 9 is also a carrier of haemophilia A which is theoretically conceivable

Definite carriers (19) of haemophilia A are III 4, IV 5 and V 9 III 4 is dead IV 5 who is in the menopause had a normal AHF which is wholly compatible with her status as a carrier (19) V 9 has had low AHF on one occasion and normal on another The labora

Table 4 *Ability to neutralise inhibitor of AHF*

		IU/ml
Haemophilia A	VI 7	0 00 −0 02
von Willebrand s disease		
Mild	V 11	0 19
Severe	VI 9	0 00
Conductors of haemophilia A	IV 2	0 22
	IV 5	0 21
	V 3	0 08
	V 9	0 08
	V 10	0 03 0 09
	VI 8	0 11
Healthy members of the family	IV 1	0 43
	IV 3	1 23
	IV 9	0 51
	V 2	0 13
	V 4	0 16
	V 5	0 16
	V 6	0 13
	VI 1	0 16
	VI 5	0 11
	VI 6	0 11
Pooled normal plasma		0 24 0 29 0 11

Table 5 *Immunochemically determined AHF*

V 9	100
V 11	92
VI 7	145
VI 9	64

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content of the AHF related protein One might imagine that in patients belonging to this group the von Willebrand factor is not lacking but abnormal with consequent formation of an abnormal complex with low procoagulant activity, with no ability to neutralise an inhibitor or to normalise the prolonged bleeding time

The relationships in the family are such that VI 9, who has severe von Willebrand s disease might also be a carrier of haemophilia A The existence of such a carrier state can be proved only if she gives birth to a child with haemophilia If she is a carrier, part of her AHF may be malformed which would further accentuate the abnormality of the AHF von Willebrand factor complex

The varying expressivity of the mother and daughter with von Willebrand s disease is however difficult to explain The mother who could neutralise the inhibitor may have a certain amount of normal von Willebrand factor or a less pronounced defect of the von Willebrand factor But the expressivity may also be influenced by other factors

In the family described there are thus genes for two rare bleeding diseases in members of the same sibship The literature contains numerous reports of remarkable combinations of prolonged bleeding time and coagulation defect and of combined coagulation defects The findings in this family suggests a reasonable explanation for some of these cases namely occurrence of two bleeding diseases

SUMMARY

A Swedish family with cases of both von Willebrand s disease and haemophilia A is described A boy has haemophilia A of moderate severity while one of his female cousins has severe von Willebrand s disease There are further cases of both diseases in the family as well as several carriers of haemophilia A The relationships of the members of the family have been checked serologically The plasma was studied for AHF related antigenic material with the aid of a human inhibitor of AHF and with the aid of a specific rabbit antiserum The patient with haemophilia appears to have a malformed AHF, demonstrable with rabbit antiserum but without ability to neutralise the inhibitor The patient with severe von Willebrand s disease has also an immunologically demonstrable AHF Tentative explanations are offered

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Fig 1 Ca e D B at 21 days of age

At 3 weeks an eryth matous indurated area about 0 5 cm in diameter was noted in th left groin Pus was not found in either of thes two lesions and no granulocytes were seen in the slight serous discharge A swab from the umbilicus grew a haemolytic streptococcus D-spite continuous antibiotic therapy (see Fig ?) the umbilical sepsis and fever continu-d until death

At 5 weeks of age the abdomen suddenly became distended due to both hepatic enlargement and ascites and at this tim the serum bilirubin was 5 3 mg/100 ml and the serum glutamate oxalate transaminase 335 units Repeated paracenteses during the remainder of his life removed about 2 litres of clear bile stained fluid The baby gradually became cachectic and died on 6th May at the age of 11 / weeks

Family history

Both parents are healthy and unrelated Th father was aged 40 and the mother 29 at the time of conception. The only abnormality detected in eith r parent was a slight eosinophilia of 450 to 700/mm In the fath r this eosinophilia was present on three of the four occasions his blood was tested In the mother the eosinophilia was noted on two of four occasions There are two normal sibs one older and the other younger than D B

Investigations

A nearly complete agranulocytosis persisted from the first blood counts when 6 days old to the time of death (Fig 2) A very occasional neutrophil was seen and in 27 differentials (-700 cells counted) only 3 neutrophils were noted This was associat d with a low grade eosinophilia (up to 1 400 mm³) and a marked monocytosis (up to 5 000 mm³) The lymphocyte count was usually between 4 000-7 000 mm The haemoglobin level was persistently low and a transfusion was required wh n 26 days old The platelet count was initially normal These findings are summarised in Fig 2 No maternal leucocyte agglutinins were pres ent Immunoglobulin levels stool trypti activity and urinary excretion of amino acids were all nor mal

The bon marrow was examined on two occasions and was of increased cellularity No neutrophil granu locyte precursors beyond the promyelocyte stage were present. The marrow differential is shown in Table 1 A numb-r of the promyelocytes were morphologically abnormal with prominent cytoplasmic vacuolation (Fig 3) The marrow revealed a marked lymph ocytosis with increases in both eosinophils and plasma cells Small cl ar vacuoles were present in some of the plasma cells

Electron microscopy of the bone marrow showed that many of the eosinophilic granules were abnormal The granules were ring shaped with pale central areas (Fig 4) and the normal crystal structure was not seen The vacuoles in the plasma cells were also demonstrated (Fig 5) Much granular material was present in macrophages (Fig 6) In several areas of the marrow collagen fibres were pres-nt (Fig 7)

Direct chromosome analysis of bone marrow cells failed to reveal analysable mitoses on two occasions Stimulation of peripheral blood lymphocytes by phytohaemagglutinin gave normal chromosome findings

Serum B_{12} was measured by *E gracilis* assay (1) The B_{12} level was 208 pg/ml when the serum was heated prior to assay and 105 pg ml without heat treatment B_{12} bound to TCI only becomes available to microbiological assay organisms after destruction of TCI by boiling B attached to the β globulin binder (now known as TCII) can be assayed without boiling (15) The unsaturated B binding capacity (10) was 445 pg/ml Separation of B binding proteins by gel filtration on Sephadex G 200 was und rtaken using the method of Hom et al (13) The results of the separation are shown in Fig 8 No definite binding of added radioactive B (Co cyanocobalamin) was seen in the position occupied by th TCI and foetal binders (peak at tube no 70) which are not separable by gel filtration (19)

Post mortem Findings

The body was that of a wasted infant Th re was much bil- tained fluid in the p ritoneum with many

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INFANTILE GENETIC AGRANULOCYTOSIS ASSOCIATED WITH CHANGES IN SERUM VITAMIN B_1 BINDING PROTEINS

M E J BEARD P NEWMARK M E SMITH and A W FRANKLIN

From the Departments of Haematology and Child Health
St Bartholomew s Hospital London England

Neutropenias occurring during the neonatal period have been recently reviewed by Kauder & Mauer (16) One of the most serious forms of such neutropenias infantile genetic agranu locytosis was first described by Kostmann in 1956 (18) In this condition there is a severe defect in the production of neutrophil granu locytes the affected children presenting with bacterial infections during the first few weeks of life and often dying in infancy

There are two main vitamin B_1 binding proteins in serum (11) an α globulin, transco balamin I (TCI) and a β globulin transcobala min II (TCII) In normal adult serum nearly all the endogenous vitamin B_1 is bound to TCI and only a small fraction is carried on TCII TCII however has a considerable capac ity to bind added B_1 whereas the binding capac- ity of TCI is almost completely saturated Early studies on serum showed that both the endoge nous B_1 level and the unsaturated vitamin B_1 binding capacity were elevated in condi tions of granulocyte proliferation (2 24) These findings suggested that TCI was a break down product of granulocytes and the pre sence of B_1 binding protein in these cells was demonstrated by Mollin & Ross (24) Meyer et al (23) subsequently showed that mature neutrophil granulocytes contained the highest concentration of this protein Radioisotope studies in vitro have confirmed that a TCI like protein can be synthesised in granulocytes (7 29) but recent evidence suggests that it may be secreted into the plasma from the intact cells rather than occur as a breakdown product (5 7) In addition to TCI and TCII a third vita min B_1 binding protein has been described in cord and neonatal serum by Kumento et al (20) and Kumento (19) This appears to be an α globulin of similar molecular weight to TCI but by contrast does not carry endogenous B_1 and thus its B_1 binding capacity is un saturated It probably disappears from the plasma within a few weeks of birth

In this paper the clinical course of a case of infantile genetic agranulocytosis is de scribed In view of the severe defect in granu locyte production serum B_1 binding proteins were analysed Electron microscopy of bone marrow cells was undertaken in an attempt to define more precisely the morphologic changes present in the granulocyte cells in this disease

Clinical summary

D B was born on 15th February 1969 following a full term gestation and normal pregnancy and delivery The birth weight was 3 4 kg He progressed normally until the 6th day when a small area of erythema was observed around the umbilicus Apart from a rectal temperature of 38 5 C (101 F) the physical examina tion was normal Over the next few days the erythema spread and was accompanied by induration of the surrounding skin extending to 5 cm in diameter (Fig 1) The umbilicus remained dry These signs presented a most unusual appearance quite unlike that found even in severe purulent infections of the umbilicus



Fig 3 Marked vacuolisation of promyelocytes The finer vacuolisation present in plasma cells is indicated by the arrow 1 500



Fig 4 Eosinophil showing abnormal granules 15 000

weeks or months of life were the presenting features and in most cases the affected children died during the first two or three years of life Examination of blood and marrow in several of these children revealed a severe neutropenia with an arrest of neutrophil



Fig 5 Plasma cell showing endoplasmic reticulum and inclusions. 15 000



Fig 6 Macrophage containing granular material 10 000

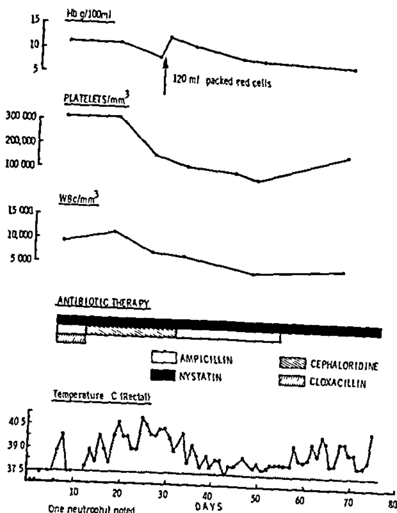


Fig 2 Clinical course of case D B

fibrinous adhesions The cells in the peritoneal exudate were almost entirely mononuclear cells and eosinophils but a very occasional neutrophil granulocyte was seen The liver was enlarged (340 g) and showed extensive centrilobular parenchymal necrosis fibrosis and calcification No thrombi were present in the main hepatic veins but there was extensive occlusion of small hepatic venous radicles and this presumably caused the liver cell necrosis Recent thrombi were also present in the small pulmonary arteries epididymis thyroid retroperitoneum and renal veins The spleen (31 g) and left inguinal lymphnodes were enlarged The other lymphnodes were normal in size and the thymus was noted to be atrophic The retroperitoneal lymphnodes exhibited severe lymphoid cell depletion but the inguinal nodes and the spleen were normal except for a small area of necrosis in one inguinal node No definite bacteria could be identified with any of the tissues examined

COMMENT

Infantile genetic agranulocytosis was first described by Kostmann in 1956 (18) In his series skin and respiratory infections during the first

Table 1 *Marrow differential count (1000 cell expressed as a percentage*

	Case D B	Control normal values
Myeloblast	1 5	0–2
Promyelocyte	1 5	0–4
Myelocyte	None	1 5–13 5
Metamyelocyte	None	4–31
Segmented neutrophil	None	2–27
Eosinophil myelocyte	3 5	0–1
Eosinophil metamyelocyte	3 5	0–1
Segmented eosinophil	1 5	0–1
Segmented basophil	0 2	0
Proerythroblast	None	0–0 5
Early normoblast	1 0	0–1 5
Intermediate normoblast	2 5	0–3 5
Late normoblast	4 0	1–15
Plasma cell	0 5	0
Lymphoblast	2 5	0 5–3
Prolymphocyte	4 0	—
Lymphocyte	69 8	27–73
Reticulum cell	4 0	0

[a] Gairdner et al (8)

ter ingestion of alcohol (25) riboflavin defi ency (21) and phenylalanine deficiency (6) o information was obtained from the elec on microscopy studies concerning the nature f this granulocyte vacuolisation Abnormal ranules were present in some but not all osinophils and none of the eosinophil gran les contained the characteristic crystals

Chromosome findings have been reported in nly one previous case described as infantile genetic agranulocytosis (22) There must be ome doubt over the diagnosis in this case ince the 37 day old infant had a severe pan cytopenia and was thus quite unlike the cases described by Kostmann Direct bone marrow chromosome study revealed many abnormali ties but examination of peripheral blood lymphocyte chromosomes was not reported In our case direct marrow chromosome analy sis failed but chromosome analysis of periph eral blood lymphocytes was normal Schroe der & Kurth (28) grouped Kostmann s Agra nulocytosis together with Fanconi s Anaemia as conditions in which a high incidence of chromosome breakage is known to occur This seems premature since the evidence for this is based on the above report (22) and furthermore in our case lymphocyte chromo somes were normal in contrast to the many abnormalities found in Fanconi s Anaemia

In normal adult sera TCI is largely saturated with endogenous B_1 and on addition of radio-active B_1 *in vitro* 50–90 of the radioactivity binds to TCII (12) However in neonatal se rum as little as 10 of the added radio activity may bind to TCII (19) the rest being bound to the TCI and/or foetal binders The serum from case D B had a low unsaturated vitamin B_1 binding capacity and all the added radioactive B_1 was bound to TCII (Fig 7) thus indicating the absence of any foetal bind er and the absence of any unsaturated TCI The serum B_1 level of case D B (208 pg/ml) was below the normal range for a child of that age (17) and about 50 of the serum B_{12} could be assayed without boiling the se rum Thus the amount of TCI present in the serum of D B has a total vitamin B_1 bind ing capacity of only about 100 pg/ml

The results in the present case show that there is probably a total absence of foetal binder and a marked reduction of TCI in the serum This is consistent with the current hypothesis that serum TCI is largely derived from mature neutrophil granulocytes and furthermore provides indirect evidence that the foetal binder may be derived from the same cells The small amount of saturated TCI present may have been derived from monocytes as these cells have been shown to contain a B_1 binder indistinguishable from that in granulocytes (4) It is unlikely to have come from eosinophils (23) The presence of a relatively large proportion of serum vitamin B_1 which could be assayed without boiling may be a result of liver cell necrosis which can lead to release of free B_1 or TCII bound B_1 into the serum (14 26)

SUMMARY

A case resembling infantile genetic agranulo-cytosis is reported A nearly complete absence of neutrophil granulocytes in the peripheral blood was associated with an arrest of neutro phil granulocyte formation at the promyelo cyte stage in the bone marrow Electron micro scopy of bone marrow revealed abnormalities of both eosinophils and plasma cells Studies of vitamin B_1 binding proteins showed a marked reduction of TCI and/or foetal bind ers

The significance of these findings is dis cussed They provide indirect evidence that TCI and/or foetal B_1 binding proteins are derived from developing and mature neutro phil granulocytes Further studies of B_1 bind ing proteins and of bone marrow morphology should be undertaken in congenital defects of granulocyte production

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We wish to thank Professor D L Mollin for help in preparing this report Dr J D Davies for review



Fig 7 Collagen fibres in bone marrow × 15 000

granulocyte production in the bone marrow at the myelocyte–promyelocyte stage A high incidence of consanguinity was present in Kostmann s cases and he concluded that the disease was probably caused by an autosomal recessive genetic defect Subsequent reports have been reviewed by Gilman et al (9) who point out that the mechanism of inheritance was not always similar to that described by Kostmann since many occur as single cases with no other affected family members The severity of the impairment of granulocyte pro duction is variable the milder defects carry ing a better prognosis Several cases reports (3 9) include children still alive in the second de cade of life although in one instance death from leukaemia has been noted in these older children (9) The present case illustrates sev eral of the findings described in Kostmann s monograph Skin sepsis was the initial feature and this appeared during the first week of life There was selective involvement of neutrophil granulocytes, apart from a slight reduction in the haemoglobin level the other cells derived from the bone marrow were normal or in creased during the first few weeks of life A normochromic normocytic anaemia and a mild but transient thrombocytopenia then de veloped It seems likely that vascular obstruc tion to the small tributaries of the hepatic vein caused the sudden onset of hepatic enlargment with ascites It is possible that this and the terminal intravascular thrombosis were con sequential to the umbilical sepsis and peritoni tis No macroscopic abscesses were present at the time of death and this is an unusual feature but the appearances at post mortem were probably modified by the continuous antibiotic therapy and the severity of the gra nulocyte production defect

Several morphological abnormalities of blood and bone marrow cells were described by Kostmann The presence of eosinophilia and monocytosis is a common finding In the marrow there is usually a marked lymphocyto sis The granulocyte precursors are frequently abnormal and Kostmann recorded that pro myelocytes and myelocytes were often atypical with lobed and vacuolated nuclei Vacuolation may also affect the cytoplasm and this is illus trated in Fig 3 This vacuolation is similar to that seen in chloramphenicol toxicity (27)

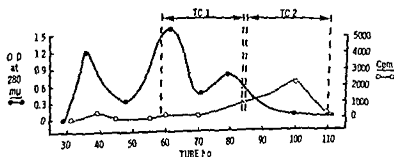


Fig 8 Separation of vitamin B bind ing proteins

after ingestion of alcohol (25) riboflavin deficiency (21) and phenylalanine deficiency (6) No information was obtained from the electron microscopy studies concerning the nature of this granulocyte vacuolisation Abnormal granules were present in some but not all eosinophils and none of the eosinophil granules contained the characteristic crystals

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SUMMARY

A case resembling infantile genetic agranulocytosis is reported A nearly complete absence of neutrophil granulocytes in the peripheral blood was associated with an arrest of neutrophil granulocyte formation at the promyelocyte stage in the bone marrow Electron microscopy of bone marrow revealed abnormalities of both eosinophils and plasma cells Studies of vitamin B_1 binding proteins showed a marked reduction of TCI and/or foetal binders

The significance of these findings is discussed They provide indirect evidence that TCI and/or foetal B_1 binding proteins are derived from developing and mature neutrophil granulocytes Further studies of B_1 binding proteins and of bone marrow morphology should be undertaken in congenital defects of granulocyte production

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INCIDENCE OF GLYCOGEN STORAGE DISEASE IN SWEDEN

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Very few studies have been presented of the incidence of glycogen storage disease (GSD) These studies comprise only relatively small series of cases from a limited population and there are discrepancies in the results (8 12–13 18)

The highest known incidence has been found in Israel in non Ashkenazi Jews of North African extraction One case in 5 420 living births has been noted in this population (8 17) Most of these cases are type III In Norway an incidence of 1/68 000 was seen (12) Most of the Norwegian cases were type III and the prevalence was two or three times higher in the Bergen and North West Coast area than in Norway as a whole A much lower figure 1/246 000 for all types of GSD was found in a study of all known Swedish cases during the period 1936–1964 (13) It is probable that the situation in Israel is unique

Although the incidence is unknown in other countries it is most probably much lower than in Israel If not so many more cases would have been found in the centers studying GSD in USA (7) Belgium (2) and also in France (11) Great Britain (10 16) the Netherlands (1) Norway (18) and Sweden (13)

More information is available on the relative frequency of the different types of GSD In both of the two large series of cases published about 25 are type I 20 are type II 25% type III and most of the remaining cases are type VI (2 3 6 7) As for incidence different results have been published for the relative frequency of the various types In Israel (8) and in Norway (12 18) GSD III dominates Also in the smaller series from France (11) and the Netherlands (1) GSD III is more common than other types In the Netherlands (5) also GSD VI a (deficiency of phosphorylase kinase) has been frequently seen In the small series from Sweden (13) GSD I was the dominant type In a recent series from Great Britain there was no case of GSD II (16)

Enzymatic diagnosis of GSD has been available in Sweden for about 10 years A re-evaluation of the incidence of GSD in Sweden can now be made together with a study of the relative frequency of the various types

MATERIAL

Some of th cases included in the present study have been referred to th author for diagnosis Some have become known when systematically searching in Swedish pediatric ho pitals for all cases with a diagnosis of GSD or in whom GSD has been susp cted It cannot b considered certain that all patients born in 1970 or 1971 have shown such symptoms and signs that a diagnosis of GSD has been looked for Therefore only patients born in 1969 and earlier are included in the study of the incidence

All cases included were native Sw des A few cases in children of immigrants (from Turkey Gre ce and Poland) are not included

METHODS

Diagnostic criteria

In all cases in whom GSD has been suspected a detailed study has been made of all relevant symptoms signs blood-chemical levels microscopy and glycogen and enzyme levels in liver and/or muscle biopsy In

Table 1 *Cases of GSD in Sweden according to type*

Type	Present study 1972	1965[a]
I	10	8
II	2	1
III	9	2
IV	—	—
V	—	—
VI[b]	9	2
Unclassified	3	—
Total	33	13

[a] From Öckerman 1965 (12)
[b] Including one case of phosphorylase kinase deficiency
[c] All these are of the hepatomegalic type

all cases the investigation has been completed until a definite diagnostic conclusion has been reached In a few cases no definite conclusion has been possible These are not included In three cases a diagnosis of GSD has been made but a definite type diagnosis has not been possible

These principles mean that the diagnosis has been made on the total amount of information available not only on biochemical findings on a biopsy sample The diagnostic criteria thereby are the same as those used earlier (13)

Glycogen and enzyme assays

The technique used for biopsy sampling and assay of glycogen and enzymes have been described earlier (9 13–15)

RESULTS

Thirty-three cases of GSD have been found in Sweden Types I III and VI are seen in about equal numbers (Table 1) Only two cases of GSD II have been diagnosed and no other types have been found Compared to the situation in 1965 (13) there is now a more equal distribution between the three hepatomegalic types In the three unclassified cases a definite diagnosis of GSD could be settled but enzyme assays have not been made or could not give a certain type diagnosis

Table 2 *Cases of GSD in Sweden according to year of birth*

Period	Present study 1972	1965[a]
1931–40	1	1
1941–50	5	4
1951–60	7	7
1961–70	18[b]	1
1971	2	—
Total	33	13

[a] From Öckerman 1965 (12)
[b] Four of these cases were born in 1960–64 but were not known when ref (12) was written

More than half of the cases were born during the period 1961–70 (Table 2) It is remarkable that four cases, who were born during the period 1960–64, had not been diagnosed when the first review of Swedish cases was written early in 1965 (13) For that reason it is possible that more cases born in the past two or three years may be found For the calculation of the incidence of GSD only the years 1961–69 are, therefore included During these nine years enzyme diagnosis was available in Sweden and all patients born during the period are two years old or more Eighteen cases of GSD could be diagnosed who were born in Sweden during these years implying 1/57 500 live births

DISCUSSION

Finding the true incidence of GSD is very difficult All the GSD s are extremely rare it is very difficult to make a definite diagnosis and methods suitable for population screening do not exist Some cases have few symptoms and may escape diagnosis Some cases are serious and die early and may also escape diagnosis

The present study does not suffice for a correct conclusion on the incidence of various types of GSD or even GSD in total It seems clear however that the availability of enzymatic diagnostic service increases the number of diagnoses made A similar effect was noted by Moe et al (12) This effect may explain the higher incidence in the present study compared to that in 1965 (13) Still the figure 1/57 500 for all types together implies that the GSD s are extremely rare disorders, much more rare than many other inborn errors of metabolism

The present study may with somewhat less uncertainty be used to conclude that the relative frequency in Sweden of the various types is not very different from that in most other series (1–4 6 7 10 11 16) with the exception of those from Israel (8 17) and Norway (18)

SUMMARY

Thirty three cases of glycogen storage disease (GSD) were found in Sweden Ten of them were GSD I two were GSD II nine were type III nine type VI and three were not classified Eighteen of the patients were born in 1961–69 implying an incidence during that time of 1/57 500 live births

It is concluded that GSD is more common in Sweden than was earlier believed but much less common than in Israel a country with a uniquely high incidence of GSD The frequency of the various types of GSD in Sweden was similar to that found in the two largest series of cases published from USA and Belgium

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GLYCOGEN STORAGE DISEASE IN NORWAY

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A very high incidence of glycogen storage disease (GSD) has been reported in Israel among Jewish immigrants from North Africa (17) and a comparatively low incidence in Sweden (26). There also seem to be differences in the incidence of different types of GSD (17).

The present paper reports the incidence, prevalence and type distribution of GSD in Norway. An attempt has also been made to evaluate the course of the disease.

MATERIALS

The first twenty cases of GSD were collected by contacting different departments at the University Hospitals in Norway and by sending an inquiry to all departments of paediatrics and internal medicine in Norway (25). A further four cases have been traced during the last 4 years.

Thirteen of the patients have been examined by us and two in the Neurological Department. Biochemical analyses have been performed on blood cells from 4 of the other 9 patients.

Two cases of probable GSD in families with type III GSD (25) are also included.

METHODS OF STUDYING TISSUE AND BLOOD CELLS

Biochemical analyses were performed on biopsy specimens from 7 of the patients and autopsy material from one. Six of the cases have been reported previously (Table 3). Biochemical analyses on liver biopsies from the other 2 cases were performed by Hers et al, Louvain and Öckerman, Lund. The methods used for these tissue studies have been described by the two authors (3, 26).

Biochemical analyses were performed at the Department of Biochemistry, University of Bergen, on erythrocytes and/or leucocytes from 14 patients. Heparinized blood samples were sent to the laboratory in ice cooled thermobags. The methods used for determination of amylo-1,6-glucosidase and glycogen contents of erythrocytes have been reported previously (25).

The absorbance spectrum of glycogen in the presence of iodine was determined according to Hers et al (5) using a polysaccharide concentration of 75 μg/ml instead of 100 μg/3 ml. The length of the outer chains of the glycogen molecule was expressed semiquantitatively by the ratio A[illegible] A[illegible].

The leucocytes were isolated by the film technique of Huijing (9) and then NaF washed and homogenized.

Phosphorylase activity in the crude homogenate was determined in the direction of glycogen synthesis using a principle described by Öckerman (27) combined with the buffer system of Yunis & Arimura (28) and the original method of Itaya & Ui (15) for the determination of phosphate liberated from Glc-1-P. Reagent mixture without homogenate was used as a blind reference.

Phosphorylase activity was also determined in the 14 000 g supernatant of the leucocyte homogenate in the direction of glycogen degradation by coupling to phosphoglucomutase and glucose-6-phosphate dehydrogenase (9).

Phosphorylase b kinase in the crude homogenate was assayed by its properties of activating added rabbit phosphorylase b (10). The AMP independent phosphorylase a activity formed from phosphorylase b by the kinase was finally determined in the direction of glycogen synthesis by assay of the phosphate liberated from Glc-1-P (28).

Protein in the leucocyte homogenate and the 14 000 g supernatant was determined by the Folin method (18).

CLASSIFICATION

Table 1 shows a classification of the 24 definite and 2 probable cases of GSD according

Table 1 *Cases of GSD traced in Norway*

GSD type	No of cases	No of families	Reported previously No of cases	Authors
I	1	1		
II	1	1	1	Kluge (16)
III	13		6	
III probable	2	8	2	Waaler et al (25)
V	2	1	2	Skullerud (21)
VI	2	2	1	Høyer & Moe (13)
VIa	2[b]	1		
Unclassified	3	3	1	Sundal (22)
Total	26	17	13	

[a] Not studied
[b] Only one of the two double first cousins studied

to the enzyme deficiency causing the disease They belong to 17 families A specific enzyme defect was demonstrated in 20 of the patients Enzyme studies were not performed on the other 6 patients In one of them glycogenosis was found in a double first cousin of a case in whom a specific deficiency had been demonstrated The case is most probably of the same type as that of his close relative studied The two probable cases of GSD belong to families with type III GSD

CLINICAL AND LABORATORY STUDIES

Table 2 shows some clinical data of the 26 patients The diagnosis of GSD was established with a high probability on the basis of clinical and laboratory studies in the first three years of life in all but one of the cases with types I III VI and VIa Clinical and laboratory findings haxe been reported in detail in two of the cases (13) Only enzymatic studies were performed in Case 3 a 23 year-old male He had been in hospital at the age of 5 years because of an enlarged liver

Ten patients (Cases 4 8 9 10 12 13 15 18 23 and 24) all showed normal ability to convert galactose to glucose

The infant with generalized GSD and the two adults with type V have been described in detail elsewhere (16 21)

The three cases with unclassified types of GSD were all subjected to careful clinical and laboratory studies All three had hepatic glycogenosis Two of them died in infancy The third patient was described in detail by Sundal in 1937 (22)

The two cases of probable GSD in families with type III GSD both died in infancy one of them in hospital at the age of 6 months She had an enormously enlarged liver and died from respiratory infection The other one was reported as having an enlarged liver and ascites Pedigrees of families 3 6 7 and 8 have been published elsewhere (Family B C A and D (25)) Case 13 in family 3 is a younger brother of Case 12 There were 6 pairs of siblings among the 26 cases Case 14 is distantly related to family 7

BIOCHEMICAL ANALYSES OF TISSUE AND BLOOD CELLS

Table 3 shows a survey of the biopsies and autopsy studies performed in 8 patients and Tables 4 and 5 the results of biochemical analyses performed on erythrocytes and/or leucocytes from 15 patients

Type I (Von Gierke s disease) Only Case 1 is considered to be deficient in glucose-6-phosphatase Biochemical analyses of liver biopsies showed high glycogen content complete lack of glucose-6-phosphatase activity and a slight increase of phosphorylase and amylo 1 6 glucosidase activities The leucocyte phosphorylase activity was relatively high Erythrocyte studies were not performed

Type II (Pompe s disease) Autopsy findings in Case 2 revealed typical findings of generalized GSD (16)

Type III (Cori s limit dextrinosis) Cases 3–15 are deficient in amylo-1 6-glucosidase Erythrocyte studies were performed in all 13 cases and biopsy studies in Cases 8 and 9 Genetic studies have been performed in four of the seven families (25) In family 3 some

Table 2 *Survey of the 24 definite and 2 probable cases of GSD in Norway*
Some of the symptomless had enlarged liver

Case no	Sex	Family no	Type GSD	Age at diagnosis (y)	Follow up in the autumn 1971: Age (y)	Height (cm)	Per centile	Comments
1	F	1	I	2	3	82	4 cm<2 5	Dyspnoea enlarged abdomen
2	F	2	II	6/12				Died 6 months old in 1964
3	M	3	III	23	27	177	>50	Symptomless married 1 child
4	F	4	III	20/12	23	171	>50	Symptomless married pregnant
5	M	4	III	18/12	20	184	>50	Symptomless student
6	M	5	III	14/12	16	168	50	Symptomless
7	M	5	III	7/12	13	130	9 cm<2 5	Symptomless
8	M	6	III	18/12	12	127	8 cm<2 5	Marked mental retardation
9	M	7	III	28/12	6½	114	10	Symptomless
10	M	8	III	21/12	5	103 5	1 5 cm<2 5	Symptomless
11	M	6	III	4/12	4½	103 5	2 5	Symptomless diabetes mellitus
12	M	3	III	9/12	3	87	1 cm<2.5	Symptomless (enlarged abdomen)
13	M	3	III	3/12	1	72	1 cm>10	Symptomless (enlarged abdomen)
14	F	9	III	18/12	2	80 5	10	Symptomless
15	F	10	III	24/12	2	87	>2 5	Tendency to vomiting enlarged liv
16	F	11	V	45	52	170	>50	Muscular weakness on exercise
17	M	11	V	26	33	170	10	Muscular weakness on exercise + peripheral nerve lesion
18	M	12	VI	15/12	6½	111	2 5	Symptomless
19	M	13	VI	9/12	6½	115 5	10	Symptomless
20	M	14	VIa	18/12	14	153 5	3 cm>10	Symptomless
21	M	14	VIa	5/12	6½	113 5	2 cm~2 5	Symptomless
22	F	15	Unclass	12	15	(130 5)*	19 5<2 5	* Not traced in 1971
23	F	16	Unclass	4/12				Died 14 months old in 1964
24	M	17	Unclass	24/12				Died 3 years old in 1965
25	F	3	Prob III					Died 15 months old in 1950
26	M	7	Prob III					Died 9 months old in 1927

additional enzymatic studies were performed (21)

Type V (McArdle s disease) The two adult siblings Cases 16 and 17 with deficiency of phosphorylase activity in muscle have been reported by Skullerud Department of Neurology University of Bergen (21)

Type VI (Hers disease) Biochemical analyses of liver biopsies from Cases 18 and 19 showed lack of phosphorylase activity Phosphorylase kinase activity was not determined at that time and they are therefore only classified as type VI

Type VIa Leucocyte studies in Case 20

Table 3 *Biochemical analyses performed on biopsy specimens or autopsy materials*

Case no	Family no	Tissue from	Place of studies	Conclusion (Type)	Reported previously (Author)
1	1	Liver	Öckerman Lund	Probably I	Not
2	2	Autopsy	Rikshospitalet Oslo	II	Kluge (16)
8	6	Liver	Hers et al Louvain	III	Waaler et al (25)
9	7	Liver + muscle	Hers et al Louvain	III	Waaler et al (25)
16	11	Muscle	Bergen	V	Skullerud (21)
17	11	Muscle	Bergen	V	Skullerud (21)
18	12	Liver + muscle	Hers et al Louvain	VI	Høyer & Moe (13)
19	13	Liver	Hers et al Louvain	VI	Not

Table 4 *Biochemical analyses performed on erythrocytes from 14 patients*

Case	Family	Amylo-1 6-glucosidase (Units/g Hb)	Glycogen (μg/g Hb)	Iodine spectrum (A[illegible]/A[illegible])	Previous no (25)	Comments
3	3	0 0	365		B-0	
4	4	0 36	191	0 86		Siblings (4, 5)
5	4	0 0	208			
6	5	0 0	370	0 88		Siblings (6, 7)
7	5	0 0	355	0 93		
8	6	0 0	790		C25	Biopsy – Tab 3
9	7	0 12	311		A19	Biopsy – Tab 3
10	8	0 12	264		D13	
11	6	0 05	430	0 92	C27	Brother of C25 – no 8
12	3	0 2	1 040		B22	
13	3	0 33	240	1 1		Brother of Case 12 (B22)
14	9	0 0	940			Distantly related to no 9 (A19)
15	10	0 0	478			
20	14	13 0	94	3 0		Type VIa See Tab 5
Normal adults						
Mean		4 10				
Range		1 7–7 3	Below 1?0	2 2 2 5		
n		15		2		

showed low phosphorylase activity and markedly decreased phosphorylase kinase activity Table 5 also demonstrates the stimulation effect of AMP on the phosphorylase activity of Case 20 as compared to a minor stimulatory in a normal case These findings are compatible with a diagnosis of type VIa (10 11) also classified as type IX (6 7) The mother also had low activities of the enzymes in her leucocytes The patient had high and the mother normal amylo-1 6-glucosidase activity in erythrocytes

Enzymatic studies were not performed in Case 21 Clinical and laboratory studies at the age of 6 months were compatible with a diagnosis of GSD His father is brother of the

Table 5 *Biochemical analyses performed on leucocytes*

		Phosphorylase				
			Homogenate (U/g protein)[b]			
Case	Family	Supernat (U/g prot)	AMP not added	AMP added	Ratio	Phosphorylase b kinase Units
1	1			4 45		
20	14	2 63	0 41/	0 81 =	0 51	0 01
Mother of ?0	14	11	1 53/	2 26 =	0 68	0 ?0
Simultaneously analysed normal		16 5	2 61/	3 26 =	0 80	0 51
Normal adults						
Mean		21 3				
Range		13 3–40 2				0 5–1 0
n		12				2

Measured in the direction of glycogen degradation 1 U = 1 μmole Glc 1 P formed in 1 min

[b] Measured in the direction of glycogen synthesis 1 U = 1 μmole inorganic phosphate liberated per 30 min

1 U = The amount of enzymes which gives rise to 100 "synthesis units of phosphorylase a per mg of leucocyte protein of the kinase reaction mixture in 5 min (pH 6 8)

Table 2 *Survey of the 24 definite and 2 probable cases of GSD in Norway*
Some of the symptomless had enlarged liver

Case no	Sex	Family no	Type GSD	Age at diagnosis (y)	Follow up in the autumn 1971 Age (y)	Height (cm)	Per centile	Comments
1	F	1	I	2	3	82	4 cm < 2 5	Dyspnoea enlarged abdomen
2	F	2	II	6/12				Died 6 months old in 1964
3	M	3	III	23	27	177	> 50	Symptomless married 1 child
4	F	4	III	20/12	23	171	> 50	Symptomless married pregnant
5	M	4	III	18/12	20	184	> 50	Symptomless student
6	M	5	III	14/12	16	168	50	Symptomless
7	M	5	III	7/12	13	130	9 cm < 2 5	Symptomless
8	M	6	III	18/12	12	127	8 cm < 2 5	Marked mental retardation
9	M	7	III	28/12	6½	114	10	Symptomless
10	M	8	III	21/12	5	103 5	1 5 cm < 2 5	Symptomless
11	M	6	III	4/12	4½	103 5	2 5	Symptomless diabetes mellitus
12	M	3	III	9/12	3	87	1 cm < 2 5	Symptomless (enlarged abdomen)
13	M	3	III	3/12	1	72	1 cm > 10	Symptomless (enlarged abdomen)
14	F	9	III	18/12	2	80 5	10	Symptomless
15	F	10	III	24/12	2	87	> 2 5	Tendency to vomiting enlarged liver
16	F	11	V	45	52	170	> 50	Muscular weakness on exercise
17	M	11	V	26	33	170	10	Muscular weakness on exercise + peripheral nerve lesion
18	M	12	VI	15/12	6½	111	2 5	Symptomless
19	M	13	VI	9/12	6½	115 5	10	Symptomless
20	M	14	VIa	18/12	14	153 5	3 cm > 10	Symptomless
21	M	14	VIa	5/12	6½	113 5	2 cm > 2 5	Symptomless
22	F	15	Unclass	12	15	(130 5)*	19 5 < 2 5	* Not traced in 1971
23	F	16	Unclass	4/12				Died 14 months old in 1964
24	M	17	Unclass	24/12				Died 3 years old in 1965
25	F	3	Prob III					Died 15 months old in 1950
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Type VIa Leucocyte studies in Case 20

Table 3 *Biochemical analyses performed on biopsy specimens or autopsy materials*

Case no	Family no	Tissue from	Place of studies	Conclusion (Type)	Reported previously (Author)
1	1	Liver	Öckerman Lund	Probably I	Not
2	2	Autopsy	Rikshospitalet Oslo	II	Kluge (16)
8	6	Liver	Hers et al Louvain	III	Waaler et al (25)
9	7	Liver + muscle	Hers et al Louvain	III	Waaler et al (25)
16	11	Muscle	Bergen	V	Skullerud (21)
17	11	Muscle	Bergen	V	Skullerud (21)
18	12	Liver + muscle	Hers et al Louvain	VI	Høyer & Moe (13)
19	13	Liver	Hers et al Louvain	VI	Not

Table 7 *Incidence of different types of glycogen storage disease in various countries*
Based on table from Levin et al (17)

Type	Holland (23) (%)	Belgium[a] (4) (%)	U S.A – St Louis[a] (14) (%)	Sweden (26) (%)	Israel (17) (%)	Norway (Present report) (%)
I	12 5	25	27	66 7	11 5	4 8
II	6 25	20	17	8 3	11 5	4 8
III	56 25	25	21	8 3	77	61 6 (65)[b]
IV			1			
V			3			9 6
VI	25	30	31	16 7		9 6
VIa						9 6
Total no	16	Approx 110	115	12	30	21 (23)[b]

[a] Biopsy specimens or referred cases
[b] Two probable cases included

of the total population in Norway All the 12 definite cases of GSD from our intake area (Bergen and the North West-Coast) are proven by enzyme assays during the last 7 years They are still alive This gives a high prevalence of GSD 12 690 000 (21 proven cases in Israel)

Levin et al state that the high number of cases of GSD in Israel is mainly due to the "unusually high prevalence of type III GSD in Israelis of North African extraction The minimal incidence of type III GSD in Israelis of North African extraction was estimated to be 1 5 420 births There were only three suspected cases of GSD in Jews of European background This will give a probable incidence for the Ashkenazi community of a magnitude similar to that of Sweden (17) Nine of the twelve cases in our intake area were type III This disease seems to follow an autosomal recessive hereditary pattern which may partly explain both the marked racial differences in Israel and the geographic distribution in Norway There is also a high rate of consanguinity on the West Coast of Norway and consanguinity occured at least in 4 of the 6 families here with type III GSD However a high rate of consanguinity occurs also in other parts of the country Only one suspected case of GSD was diagnosed in our intake area before the studies were started in 1965 Careful search for patients suffering from GSD in other parts of Norway may disclose a similar high prevalence

Table 6 shows that type III seems to be the most frequent type of GSD in Holland Israel and Norway (56-77-62 per cent) The incidence figures from Belgium (Hers) and those reported by Illingworth may represent a selection of types of GSD since they mostly seem to refer to cases where biopsy was necessary Such a selection may omit milder cases of GSD Only one case (tentatively) of Type III among 13 cases from Sweden is exceptionally low Type VI with subgroups seems to occur somewhat less frequently than type III The two types together comprised 81 77 81 % of typed cases in Holland Israel and Norway respectively

Type I was demonstrated in one of our patients and may have been present in a maximum of 4 of 24 (17 %) definite cases of GSD Table 6 shows an incidence of type I GSD up to 27 % in Sweden 62 % (8/13)

Type II occurred in all the 6 materials Table 6 confirms that types IV and V are extremely rare

PREVENTION

Types III and VI with subgroups seem to comprise the majority of cases of GSD From a practical point of view this is very important

Table 6 *Distribution of cases of GSD in Norway*

Part of Norway	No of cases of GSD Total	Type classified	Families	Type III	Population (Approximate no Thousands)
Bergen area	8 (9)[a]	8 (9)[a]	6	5 (6)[a]	370
North west-coast	4 (5)[a]	4 (5)[a]	2	4 (5)[a]	320
South west coast	0	0		0	270
Northern	4	4	2	4	450
Middle (Trondelag)	1	0	1	0	350
East	7	5	6	0	2 200
Southern	0	0		0	210
Total	24 (26)	21 (23)	17	13 (15)	3 900

[a] Included a probable case

mother of Case 20 and his mother sister of the father of Case 20 The mother of Case 21 had normal amylo 1 6 glucosidase activity in her erythrocytes when studied in 1967

COURSE OF THE DISEASE

Table 2 shows data obtained on the 26 patients in the autumn 1971 Three definite and the two probable cases of GSD are dead and it has not been possible to trace Case 22 The child with type I still has a markedly enlarged abdomen with mild respiratory distress All but one of the 13 patients with type III and the four with types VI–VIa seem to be free of symptoms, except for an enlarged abdomen in the smallest children The liver seems to decrease in size as the children grow older The three adults seem to be doing fine they all have normal height Case 8 a 12 year old boy is markedly mentally retarded probably as a result of neurological damage caused by hypoglycaemia in infancy His brother Case 11 developed diabetes mellitus at the age of 3 years (19)

The two adults with type V still have muscular weakness on exercise The male has a peripheral nerve lesion of unknown cause Less than half of our patients with GSD seem to have retarded growth

INCIDENCE PREVALENCE AND GEOGRAPHIC DISTRIBUTION

All but one of the 24 patients with definite GSD have been studied during the last 10 years twenty two were born during a 27 year period During this period about 1 5 million infants were live born in Norway This suggests a minimal incidence of GSD of about 1 68 000 Öckerman (26) in his comprehensive study traced 13 definite or highly probable cases of GSD in Sweden all but one were classified as to type A few other patients might have had GSD and the minimal incidence was calculated to be about 1 246 000 live births A total of 49 cases of GSD have been diagnosed in a 13 year period in Israel Twenty one were proven by enzyme assays there were 3 affected siblings and the type was presumed in 6 cases No suggestion as to type was made in 19 suspected cases of GSD It is also difficult to calculate the incidence of GSD in Israel since not all the cases of GSD were born there (17)

Levin et al (17) reported a "prevalence of GSD in Israel of 49 2 400 000 compared to 13 7 650 000 in Sweden however not all the cases were alive at the same time And the two materials are not quite comparable The minimal prevalence in Norway in the autumn 1971 is 20 3 900 000 all classified as to type

A total of 17 families with GSD have been traced in Norway all of them during the last 10 years The enzyme defect has been demonstrated in 14 of the 17 families

Table 5 shows that 8 of the 24 definite and one probable case are from the Bergen area The population in this area is less than 1/10

Table 7 *Incidence of different types of glycogen storage disease in various countries*
Based on table from Levin et al (17)

Type	Holland (23) (*)	Belgium (4) ( )	USA - St Louis (14) ( )	Sweden (ˀ6) (•)	Israel (17) ( )	Norway (Present report) ( )
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II	6 75	20	17	8 3	11 5	4 8
III	56 25	25	21	8 3	77	61 6 (65)[a]
IV			1			
V			3			9 6
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Biopsy specimens or referred cases
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Type II occurred in all the 6 materials Table 6 confirms that types IV and V are extremely rare

PREVENTION

Types III and VI with subgroups seem to comprise the majority of cases of GSD From a practical point of view this is very important

partly because they represent the mild types of GSD and partly because in most cases it is easy to make a correct diagnosis by performing enzymatic assays on blood cells Heterozygotes may even be traced (1, 24, 25) and genetic counselling given

Type II may be diagnosed early in pregnancy and abortion advised (20)

Types IV and V are extremely rare and the muscular weakness on exercise in adults with type V seems not to offer a great problem This leaves type I the classical Von Gierke s disease as the major problem among cases of GSD It is not an infrequent type, the prognosis is more serious than in types III and VI with subgroups and a biopsy specimen is required for diagnosis

In all cases of hepatic glycogenosis an early diagnosis is important in order to try to prevent hypoglycemia in infancy In most patients this may be achieved by simple dietary management Lower respiratory infection must also be treated vigorously in small children with GSD

SUMMARY

Twenty four definite and two probable cases of GSD have been traced in Norway The minimal incidence is about 1 68 000 live births Thirteen of twenty-one cases are considered to belong to type III and four to type VI or VIa The prognosis seems to be good in these types and it should be easy in most patients to make the diagnosis by performing blood cell assays

The prevalence of typed cases of GSD in our uptake area is a minimum of 12 690 000 This high figure may be due to a high rate of consanguinity on the West Coast of Norway but also to a careful search for cases here

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ADDENDUM

Öckerman has now found a total of 31 cases of GSD in Sweden nine were type III (*Acta Paediat Scand* 1972)

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IMMUNOGLOBULIN TURNOVER IN A NEWBORN INFANT WITH THE CONGENITAL NEPHROTIC SYNDROME

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The serum protein pattern in the congenital nephrotic syndrome (CN) shows extreme hypo proteinaemia and dysproteinaemia (6) which is due to abundant proteinuria increased de gradation and perhaps also to enteral loss of protein (14) The total protein content in serum is usually 3–3 5 g/100 ml (range 1 4–6 0) Albumin is always very low (0 2–1 5 g/100 ml) but α2 globulins are regularly more or less elevated (0 8–5 3 g/100 ml) (6) The levels of immunoglobulins in CN are also characteris tically changed low or even absent IgG clear ly elevated or normal IgM and low or some times normal IgA (11) Using protein frac tions labeled with radioactive isotopes Per heentupa was able to make a map of the pro tein turnover in CN (14) It was stated by him that the synthesis of protein in CN is one and a half to three times larger than the normal rate The half life of γ globulin was reported to be only 8 5–23 hours and accord ingly its synthesis was 1 9–12 times larger than normally There are still no detailed data on the fate of different immunoglobulins in pa tients with CN This study was made to gain further information of this nature Proteinuria occurs even in CN fetuses (11) and so neph rotic changes are present even in cord blood (11 12) An exchange transfusion was made in a newborn CN infant to correct the protein status The serum levels of immunoglobulins were then studied repeatedly for a period of 10 days

MATERIAL AND METHODS

The patient The patient was the second child of healthy 27 year old parents The first child had succumbed to CN at the age of 3 weeks The patient under study was born two weeks before term The birth weight was 2 950 g and the weight of the placenta 1 000 g (i e 30 % of the birth weight) The large placenta gave rise to a suspicion of CN and this diagnosis was verified in the laboratory tests (urine protein ad 6 5‰ erythrocytes 50–60 and leuko cytes 10–15 per high power field serum protein 3 0 g/ 100 ml serum protein pattern typically nephrotic (Fig 1)

At the age of 5 days an exchange transfusion was made with 625 ml of fresh citrated blood The ex change transfusion was made in aliquots of 20 ml in the course of 54 min There was a negative balance of 10 ml at the end of the procedure The serum immunoglobulin levels of the patient before the exchange transfusion and those of the donor s blood were as follows

	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)
Patient			
About 2½ days before exch tr	290	0	30
Just before exch tr	110	0	35
Donor	855	212	94

Methods Samples of capillary blood were used for the serum immunoglobulin estimations except in the connection with the exchange transfusion where venous blood was available Special efforts were made to avoid incorporating oedema fluid in the blood sample Immunoglobulins were estimated by a direct immunodiffusion technique as described by Yoko yama & Yamakido (18) Sera with known levels of immunoglobulins were used as controls Cellulose acetate electrophoresis was carried out on a number of samples of serum urine and ascitic fluid Hapto globin levels were estimated as described by Taru koski (17)

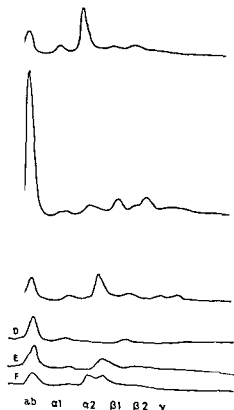


Fig 1 Electrophoretic analysis of protein patterns of plasma urine and ascitic fluid *A* Serum before exchange transfusion (ET) *B* serum immediately after ET *C* serum 9 days after ET *D* urine (protein content 7 5 g l) *E* and *F* ascites at 1 and 2 5 months respectively (concentrated tenfold from the original samples)

Later course of the disease Repeated transfusions of blood and plasma were needed after the observation period of 10 days There were frequent infections The patient succumbed to a septic infection at the age of 4 months The autopsy findings were typical of CN

RESULTS

After the exchange transfusion the serum protein pattern was totally normal (Fig 1) As is seen in Figs 2 *a b* and *d* the disappearance rates of IgG and IgA were very *similar* It became obvious that the disappearance rapidity of IgG and IgA from the blood in this CN patient depended on their concentrations in the serum (Figs 2 *a b d*) The approximate half lives of immunoglobulins at different concentrations are given in Table 1

The disappearance rate of IgM after the exchange transfusion was different from those of IgG and IgA (Fig 2 *c d*) The IgM level remained at about 100 mg/100 ml for 8 days Thereafter a rapid fall in the IgM level seems to have occurred but only one sample was available from this period During the first week after the exchange transfusion the IgM level (including the patient s own synthesis of IgM) showed a "half life of about 15 days

There was a rise in the haptoglobin levels during the second and third days after the exchange transfusion (Table 2)

At the age of one month the levels of immunoglobulins in the serum were as follows IgG 20 mg/100 ml IgA 18 mg/100 ml and IgM 57 mg/100 ml No blood or plasma transfusions were given during the last week before taking the sample The protein content in urine was 3 7–6 5‰ before the exchange transfusion and 8–21 6‰ during the first post transfusion week The proteinuria was also of a similar magnitude during the later course of the disease The protein of the urine was predominantly albumin while albumin and α2 globulins were approximately equally prominent protein constituents of the ascites fluid The protein content of the latter varied from about 100 to 500 mg/100 ml (Fig 1)

DISCUSSION

The present patient showed a very typical clinical picture and history of CN The diagnosis must be regarded as fully verified (6)

A newborn infant with CN is most suitable for studies on the turnover of IgG and IgA because its own synthesis of those immunoglobulins is still either lacking or minimal (11) After exchange transfusion the half lives of IgG and IgA in newborn infants have been reported to be about 30 and a little less than seven days respectively (13) The patient s own synthesis of IgM compensates for the catabolism of IgM during the first two weeks

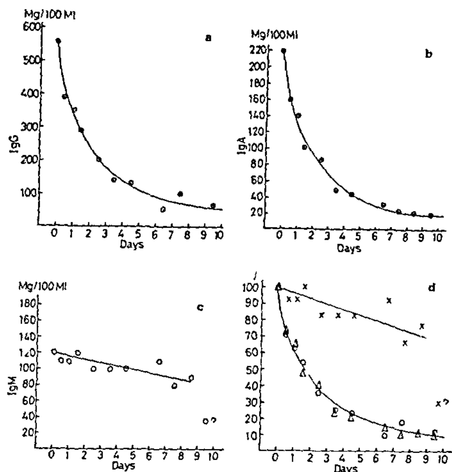


Fig. 2 a, b, c. Disappearance curves of serum IgG (*a*), IgA (*b*) and IgM (*c*) after exchange transfusion. (*d*) The daily fall of immunoglobulins in percentage of the level at the end of exchange transfusion. O IgG, △ IgA, × IgM.

after exchange transfusion (13). Synthesis of IgM in newborn infants is especially intensive in septic infections (10).

Immediately after the exchange transfusion the IgG level was 555 mg/100 ml in the case under discussion, the preexchange content being 110 mg/100 ml. Because the IgG level in the donor's blood was 855 mg/100 ml and about 70% of the baby's blood was exchanged and because IgG has fairly free access to extravascular space (9), the level after the exchange transfusion was approximately as expected. The levels of IgA and IgM were relatively higher than the IgG level. This may at least partly be due to their slower access to extravascular space. This explanation correlates well with the later behaviour of IgM but on this basis one would have expected a

Table 1. *The approximate half lives of serum immunoglobulins at different concentrations*

Ig-class	Concentration (mg/100 ml)	Half life (Days)
IgG	550	1.5
IgG	400	2.0
IgG	300	2.2
IgG	200	2.8
IgG	100	5
IgA	220	1.4
IgA	160	1.6
IgA	100	2.2
IgA	60	2.4
IgA	40	3.8
IgM	100	(15[a])

[a] Patient's own synthesis of IgM compensates partly for the catabolism of the allogeneic IgM.

Table 2. *The serum haptoglobin levels before and after exchange transfusion*

Sampling time	Haptoglobin level (mg/100 ml)
1 day before exch. tr.	17
Just before exch. tr.	14
Just after exch. tr.	26
1 day after exch. tr.	24
2 days after exch. tr.	36
3 days after exch. tr.	31
4 days after exch. tr.	21

lower initial level of IgA Some unknown fac tor possibly a technical one may have been responsible for the high IgA level in the first sample after the exchange transfusion

The disappearance curves of IgG and IgA in the present CN patient were surprisingly alike The regular parabolic form of these curves (Figs 1 *a b d*) clearly indicates that in CN the disappearance rates of these two im munoglobulins are dependent on their con centrations in serum as indicated by the approximate figures in Table 1 Providing that the fall in the serum levels of IgG and IgA is totally or mainly due to their loss in urine the results of this study indicate a constant leak of special plasma through glomerular basal membrane The "special plasma would contain proteins of smaller molecular size including both IgG and IgA The half life of plasma γ-globulin seems to some extent to depend on the concentration though that is not seen in the fractional turnover rate (1 5 9) The plasma curves of the disappearance of IgA and IgG in normal newborn infants have a very gently sloping parabolic or nearly linear *form* (13) In older CN-children despite low serum levels the degradation of γ-globulin is said to be greately *accelerated* (14)

The disappearance rate of the IgG received in utero from the mother seemed to be in ac cordance with th results of the study after the exchange transfusion There was a fall from 290 mg/100 ml to 110 mg/100 ml in about 2 5 days which in principle corresponds to the values seen in Fig 2 *a* The half lives of IgG and IgA are strikingly shorter than those for babies to whom exchange transfusion has been made on the basis of *isoimmunization* or hyperbilirubinaemia (13) However they are clearly longer than that of γ globulin reported in CN by Perheentupa (14)

The IgM level of the patient was 30 mg/ 100 ml at the age of 2 days and 35 *mg/100 ml* at the age of 5 days just before the *exchange* transfusion This is a relatively high level for the age (2) In the later course of CN a high or very high IgM level is a frequent finding (11) IgM is probably not lost in urine Forma tion of IgM antibodies after antigenic stimula tion is inhibited when IgG antibodies against the same antigen appear (4) As shown in this study and some earlier ones (11 14) IgG anti bodies are lost very rapidly in CN According ly IgM synthesis may continue uninterrupted for a longer period of time This study showed that in CN IgM antibodies remain in the body for a much longer period of time than IgA and IgG antibodies The half life of IgM in CN may even be slightly longer than that of normal newborns (13) Relatively high serum haptoglobin level may indicate infection (16) which could greatly increase the patient s own IgM synthesis (10) High level of IgM can result in tagging of this protein in glomeruli (8) Deposition of immunocomplexes from cir culation in the capillary walls of the glomeruli is greatly intensified by increased vascular permeability i e proteinuria (3) The serum levels of IgM may at least partly explain the presence or lack of immunoglobulin in CN glomeruli (6 7 11 15)

In CN there is a severe secondary defect of humoral immunity caused by short half lives of IgG and IgA This defect may be partly compensated by intensive formation of IgM antibodies In any case the relatively long half life of IgM recorded in this study makes its normal or high serum levels in CN under standable

SUMMARY

The serum immunoglobulin turnover was stud ied in a newborn infant suffering from the congenital nephrotic syndrome Serum levels of IgG IgA and IgM were estimated daily for a period of ten days after an exchange transfusion The disappearance rates of IgG and IgA were very rapid and similar to each other The higher the concentration of these proteins in the serum the shorter are their half lives During the first day after the ex change transfusion the half lives of IgG and IgA were 1 5 and 1 4 days and four days later 5 and 3 8 days respectively

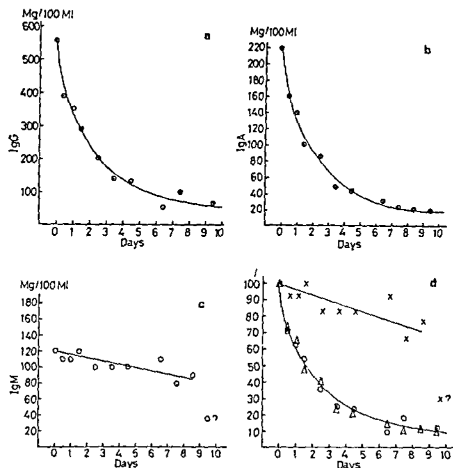


Fig 2 a b c Disappearance curves of serum IgG (*a*) IgA (*b*) and IgM (*c*) after exchange transfusion (*d*) The daily fall of immunoglobulins in percentage of the level at the end of exchange transfusion O IgG Δ IgA × IgM

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IgG	100	5
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The half life of IgM was within normal limits The secondary severe deficiency of IgG and IgA in CN seems to be partly compensated by saving of IgM antibodies

ACKNOWLEDGEMENT

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BLOOD VOLUMES IN PREMATURE INFANTS OF DIABETIC AND NON DIABETIC MOTHERS CORRELATED WITH THE TIME OF CLAMPING OF THE UMBILICAL CORD

J G KLEBE, C JOH INGOMAR and B NØRGAARD-PEDERSEN

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Newborn infants of diabetic mothers are often seen to develop clinical signs indicative of hypervolaemia Thus tachypnoea tachycardia and plethora are common findings and in some cases cardiomegaly may be visualized by X ray examination

Furthermore an increased development of the haemopoietic tissue has been demonstrated (5)

The present report concerns the blood volume of infants of diabetic mothers calculated on the basis of direct determination of the blood volume and haematocrit value The results are compared with those obtained from infants of identical gestational ages and born of non-diabetic mothers using one and the same technique for the clamping of the umbilical cord The plasma volume has been found to be lower among infants of diabetic mothers than in those of non-diabetic mothers An explanation of this finding and the importance of the placental transfusion in relation to the neonatal haemodynamic adaptation will be discussed

MATERIAL AND METHOD

The series comprises a group of infants who participated in a program of perinatal investigation of new borns at risk The investigation was performed between June 1970 and July 1971 The blood volume was measured in 54 of these cases 78% of the infants of diabetic mothers and 76% of the infants of non-diabetic moth rs were examined 1-3 hours after birth the rest being examined 4-24 hours after birth Thirty three were infants of diabetic mothers (Table 1) and 21 were infants of non-diabetic mothers (Table 2) Tables 1 and 2 show the distribution according to gestational age and birth weight Table 3 shows the mode of delivery and the maternal complications are recorded in Table 4

Early or late clamping of th umbilical cord has been randomized care being taken however that the two groups were to comprise approximately equal numbers of each type Early clamping is defined as clamping within 5 sec after presentation of the buttock and usually prior to the first cry Late clamping denotes clamping 1 5 2 min after presentation of the buttock and always after crying has begun

The blood volume was measured by means of iodinated ¹³¹J human serum albumin (Philips Duphar Ames Atomium) in which the content of free iodine determined by precipitation with trichloro-acetic acid was found to be 0 8% Doses were in all cases less than 0 5 μCi The children received 2 drops of Lugol s solution prior to examination and 2 drops administered by mouth on the following day Residual placental blood served as premix reference value As a postmix value 2 5 ml of heparinized blood was drawn 10 min after injections via a new umbilical catheter The haematocrit values (Hct) were determined in duplicate after spinning for 5 min in a microcentrifuge Taking into consideration the difference between venous haematocrit values and body haematocrit values as well as th trapped plasma th corrected erythrocyte volume (EV) and the corrected blood volume (BV) were subsequently calculated according to the formulae

$$EV_{co} = BV_{co} \times Hct_{co}$$

$$BV \quad = \frac{BV_m \times 100 - Hct_{co}}{100 - 0\ 9 \times Hct}$$

BV_m = blood volume read on Volumetron The plasma volume (PV) was calculated as the difference between BV and EV

Table 1 *Infants of diabetic mothers*

No	Gest age (weeks)	Weight (kg)	Blood vol (ml/kg)	Erythrocyte vol (ml/kg)	Plasma vol (ml/kg)	Hcrit
1	<31	1 400	86	37	49	56
1	31	1 200	88	35	53	47
3	32	2 617	78	33	46	48
		2 00-2 95	65-93	28-40	37-63	38-58
1	33	2 900	92	43	49	55
1	34	2 450	78	42	36	63
4	35	3 050	97	53	44	63
		2 60-3 55	79-112	38-66	39-51	57-72
9	36	3 447	86	44	42	60
		2 70-4 20	63-98	30-56	33-52	55-72
8	37	3 631	84	42	42	58
		2 55-4 55	61-104	29-61	37-51	46-73
4	38	3 350	94	45	49	55
		2 80-4 00	80-104	31-61	43-55	45-68
0	39	—	—	—	—	—
1	Doubtful	3 400	93	54	43	65
Total	Mean					
33	35 5	3 175	87	46	44	58

RESULTS

It appears from Figs 1 2 and 3 that the total volume of blood as well as the total volumes of erythrocytes and plasma were correlated to the weight at birth, increasing with increasing weight This is seen to apply equally well to the two groups whether the umbilical cords were clamped early or late and to the groups of infants whether of diabetic or healthy mothers

The influence of gestational age on total blood volumes was found to be less than that of weight at birth and the total blood volume was expressed as ml/kg identical at various

Table 2 *Infants of non diabetic mothers*

No	Gest age (weeks)	Weight (kg)	Blood vol (ml/kg)	Erythrocyte vol (ml/kg)	Plasma vol (ml/kg)	Hcrit
1	<31	1 300	99	41	58	48
2	31	1 625	96	42	55	50
		1 50-1 75	91-102	37-47	54-56	48-53
1	32	2 000	75	35	40	54
3	33	1 685	97	49	49	58
		1 45-2 06	85-112	38-66	46-53	52-69
2	34	2 252	101	53	49	61
		2 00-2 51	101-102	51-56	45-52	57-65
2	35	2 400	100	48	52	56
		2 25-2 65	97-103	47-49	48-56	53-59
4	36	2 512	92	42	50	53
		2 00-2 90	79-106	33-57	41-62	46-68
2	37	2 325	76	27	49	42
		2 30-2 35	74 78	22-33	46-52	35-49
0	38	—	—	—	—	—
2	39	3 200	80	35	46	52
		2 90-3 50	78-82	33-37	42-49	48-55
2	40	3 375	100	51	49	61
		3 25-3 50	90-109	50-52	40-57	56-65
Total	Mean					
21	35	2 374	92	44	49	54

Table 3 *Type of delivery*

	Vaginal delivery	Caesarean section
IDM	12	21
Non-IDM	18	3

Table 4 *Maternal complications*

	Pre eclampsia	Bleeding	Hydrorrhoea
IDM	6	6	0
Non IDM	1	6	2

Delivery induced on account of spontaneous contractions or discharge of amniotic fluid

ages of gestation In Figs 4 5 and 6 the volumes of blood erythrocytes and plasma are expressed in ml/kg and the results thus obtained were correlated to modes of delivery (vaginal/caesarean section) diagnosis (IDM/non IDM) and techniques used for the clamping of umbilical cords (early/late)

It appears from Figs 4 and 6 that modes of delivery had no decisive influence on blood and plasma volumes The erythrocyte volume however is probably influenced Fig 5 a problem to be discussed in another publication

It appears from Figs 1 and 4 that the volume of blood (ml/kg) in IDM as well as in non IDM was significantly higher in infants whose cords were clamped late than in infants whose cords were clamped early The same applies to the erythrocyte volume (Figs 2 and 5) Furthermore in infants whose cords were clamped at identical stages the volumes of blood and erythrocytes in IDM and non IDM were not signifcantly different Conversely volumes of plasma were independent of clamping techniques no matter whether mothers of the infants were diabetic or non diabetic (Figs 3 and 6) Furthermore a comparison of the two groups IDM and non IDM resulted in following findings The early clamped IDM decreased their plasma volume significantly more than the non IDM (Fig 6) In the late clamped group IDM also decreased their plasma volume more than the non IDM group but the difference was not as marked as in the early clamped group Regardless of the clamping technique a comparison of the groups showed that the plasma volume was significantly lower in infants of diabetic mothers ($p < 0\ 01$) (Wilcoxon Test)

DISCUSSION

According to the results obtained in our examinations of infants of non-diabetic mothers

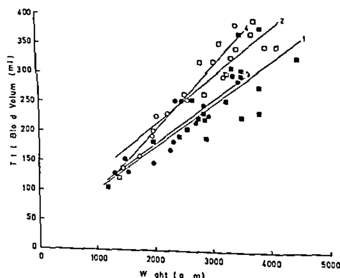


Fig 1 ■ IDM/EC regression line 1 $y = 0\ 073x + 26\ 7$ □ IDM/LC r gression line 2 $y = 0\ 080x + 50\ 3$ ● NON IDM/EC regression line 3 $y = 0\ 073x + 30\ 35$ ○ NON IDM/LC regression line 4 $y = 0\ 104x - 10\ 3$ The correlation between total b ood volume and birth weight in 33 infants of diabetic mothers (IDM) and 21 infants of non diabetic moth rs (Non IDM) in relation to early clamping (EC) and late clamping (LC) of the umbilical cord

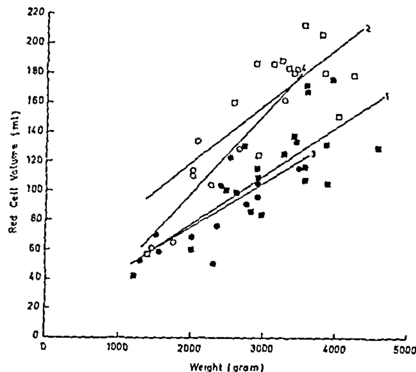


Fig 2 ■ IDM/EC regression line 1 y = 0 034x + 10 5 □ IDM/LC regression line 2 y = 0 041x + 39 7 ● NON IDM/EC regression line 3 y = 0 031x + 13 8 ○ NON IDM/LC, regression line 4 y = 0 054x − 7 5 The correlation between the total erythrocyte volume and birth weight in 22 infants of diabetic mothers (IDM) and 21 infants of non diabetic mothers (Non IDM) in relation to early and late clamping (EC) (LC) of the umbilical cord

the volumes of blood, erythrocytes and plasma were found to range at levels somewhat higher than those hitherto reported (3 and 7)

The difference in applied doses the different terms of intermixture and stages at which examinations were made, as well as the different technical methodology used and the differently denatured albumin may serve to explain this discrepancy On the other hand it was not of any consequence when findings in infants of diabetic and non diabetic mothers were to be compared Our results, however, are in fairly good agreement with those obtained by direct determination of the magnitude of the placental transfusion which amounts to about 100 ml in full term new

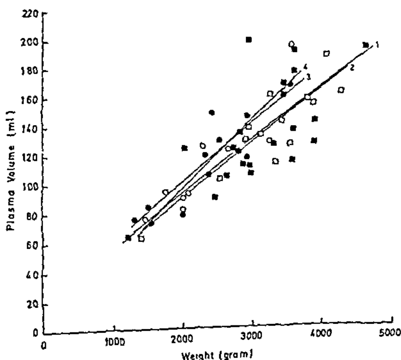
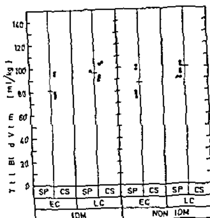


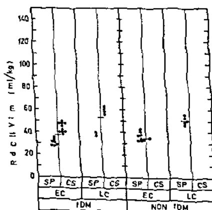
Fig 3 ■ IDM EC regression line 1 y = 0 039x + 15 8 □ IDM LC regression line 2 y = 0 040x + 10 7 ● NON IDM, EC regression line 3 y = 0 042x + 16 9 ○ NON IDM LC regression line 4 y = 0 050x − 3 1 The correlation between the total plasma volume and birth weight in 33 infants of diabetic mothers (IDM) and 21 infants of non diabetic mothers (Non IDM) in relation to early and late clamping (EC) (LC) of the umbilical cord



Blood volume ml/kg	IDM/EC	Non-IDM/LC
IDM/LC	$p<0\ 01$	NS
Non IDM/EC	NS	$p<0\ 05$

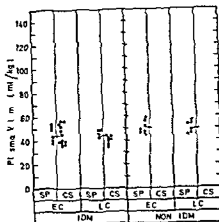
Fig 4 Rank test (two-tailed $p=2\alpha$) SP spontaneous vaginal delivery CS caesarean section EC early clamped LC late clamped IDM infant of diabetic mother The total blood volume ml/kg in infants of diabetic mothers and in infants of non-diabetic mothers in relation to mode of delivery and clamping technique

borns (1 2) The difference in erythrocyte mass per kg of body weight in cases of early and late clamping was thus found to be about 15 ml in our series As the haematocrit



Erythrocyte vol ml/kg	IDM/EC	Non-IDM/LC
IDM/LC	$p<0\ 01$	NS
Non-IDM/EC	NS	$p<0\ 01$

Fig 5 Rank test (two-tailed $p=2\alpha$) SP spontaneous vaginal delivery CS caesarean section EC early clamped LC late clamped IDM infant of diabetic mother



Plasma volume ml/kg	IDM/EC	Non-IDM/LC
IDM/LC	NS	$p=0\ 1$
Non IDM/EC	$p<0\ 05$	NS

Fig 6 Rank test (two-tailed $p=2\alpha$) SP spontaneous vaginal delivery CS caesarean section EC early clamped LC late clamped IDM infant of diabetic mother

values in cord blood are approximately 50% the placental transfusion can be calculated to be about 100 ml in infants at term Their weight at birth averaging 3 300 g

According to our findings the only statistically significant difference between infants of non diabetic and diabetic mothers was that the volume of plasma after birth adapts to a lower level in IDM no matter whether cords are clamped early or late It is generally accepted that infants whether their umbilical cords are clamped early or late regulate their blood volumes within the first hours of life the outcome being almost identical levels In infants whose umbilical cords are clamped late the haematocrit values rise a phenomenon attributed to transduction from the intravascular space to the extravascular space (6) In infants whose cords are clamped early the haematocrit values fall accordingly as absorption of fluid from the extravascular space takes place

The results obtained in the present study demonstrate that such transfer of fluid occurs within the first hours after birth

In 77 of the cases the blood volume determination was done 1–3 hours after deliv

ery, and in almost every case we noted maximal haematocrit change This indicates that the transfer of fluid, at the time of investigation had occurred In addition the results show that there is a quantitative difference in the regulation of the plasma volume between infants of diabetic and non diabetic mothers, also confirmed by the more extensive rise in haematocrit in the IDM group (Tables 1 and 2)

The fact (4) that the fatty tissue which is poorly vascularized is particularly well developed in infants of diabetic mothers might serve to corroborate the point of view that reductions in plasma volumes represent an adaptation to a relatively poor vascular capacity This hypothesis may be of value in considerations concerning the stage at which to clamp the umbilical cord

SUMMARY

In a series comprising 33 infants of diabetic mothers and 21 infants of non diabetic mothers the blood volumes were determined 1 to 12 hours after birth, using ^{131}J labelled human albumin The umbilical cords were clamped early or late It applies to both categories of infants that there was positive correlation between weight at birth and total volumes of blood erythrocytes and plasma respectively Volumes of blood and erythrocytes, expressed in ml/kg of body weight were found to be significantly higher in infants whose umbilical cords had been clamped late than in infants whose cords had been clamped early regardless of whether their mothers were diabetic or non diabetic

It applies to both groups of infants whose cords were clamped at identical stages that there was no significant difference in volumes of blood and erythrocytes per kg of body weight while plasma volumes in infants of diabetic mothers were lower than those in infants of non diabetic mothers Volumes of plasma per kg of body weight, however were found to be identical in infants in the groups IDM and non IDM respectively no matter whether their cords had been clamped early or late

The haemodynamic adaptation in the neonatal infants of diabetic mothers is in principle the same as that in infants of non diabetic mothers, but the former adjust themselves to a lower plasma volume

The hypothesis is advanced that infants of diabetic mothers immediately upon birth adapt themselves to a volume of plasma smaller than that seen in normal infants owing to the fact that the former have a smaller vascular capacity as related to their weight This in turn may depend upon their extra amount of relatively non vascular adipose tissue which adds to their weight at birth

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AUDITORY SCREENING OF FOUR YEAR OLD CHILDREN

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Children with defective hearing may be handi capped in their ability to communicate as well as in intellectual emotional and social devel opment (13)

Most severely deaf children are now detected at an early age but partially hearing children if not examined may be undetected through out pre school age (6 11) as their symptoms are not always well understood by parents or teachers (10 13)

The need to identify hearing impairment as early in life as possible is well recognized but simple screening methods with proven high sensitivity and specificity are not available until the child is some years old (1 3 7 13) To be effective a screening program of this kind must be included in the organized general health service program for children The pres ent study reports on a screening for hearing defects in 4-year old children as a part of a general health control

MATERIAL

In an effort to bridge the gap of efficient health con trol of children belonging to age group betwe n infancy and school age a comprehensive study of all 4 year-old children was started in 1967 in the city of Lund and somewhat later in the community of Dalby (located in the vicinity of Lund) All chil dren of 4 years of age living in these areas we e elected from the county population register Children li ing temporarily in the areas but regi tered el e [illegible] There was a total of 2 573 4-year-old children 2 296 living in Lund 1967–1969 and 277 in Dalby 1968–1969

METHODS

The children were invited to participate by a letter to their parents The invitation was accompanied by questionnaires regarding, *inter alia* language d velop-ment, previous otitis media present complaints of impaired hearing or of adenoidal symptoms No effort was made to confirm the information from any re cords

The screening of hearing was p rformed by specially trained nurs s at 2 Child Health Centers according to the play audiometry principle (1) using a pure tone audiometer (Tegner PTA 9) with double earphones During the first year the testing was made at 3 frequencies 250 1 000 and 4 000 cps on 634 children born in 1963 The following two years another 3 frequencies 500 2 000 and 8 000 cps were also used on 1 780 children born 1964–1965 A level of 20 dB ISO was considered normal except at 250 cps where 25 dB was accepted becau e of th unavoidable en vironmental noise in the testing room tending to mask mainly the lower frequencies Children who did not perceive the test tones were prescribed with nasal decongestants and were re tested 2 3 weeks later Those who again did not hear th test tones were re ferred to the Department of Audiology for further investigation

At the paediatric examination performed on the same day as the first hearing screening special con sideration was taken to signs of adenoids (snoring open mouth thick speech) These symptoms in com bination with a history of frequent upper respiratory infections and/or otitis media or pronounc d mecha ni al interference were an indication for reference to the otologist irrespective of the results from the hearing examination

At the Department of Audiology a complete audio-logical evaluation together with a full examination of ears nose and throat was performed Otological and audiological treatments were institu ed if necessary

Table 1 *Main otologic diagnosis of 133 referrals from auditory screening of 2414 four year old children*

	n	of referred (n = 133)	of screened (n = 2 414)
Conductive hypoacusis	89	66 9	3 7
Middle ear infections	73	54 9	3 0
Mild infections	37	27 8	1 5
Severe infections	36	27 1	1 5
Cerumen	16	12 0	0 7
Sensorineural hypo acusis	5	3 8	0 2
Normal findings	30	22 5	1 2
Not examined	9	6 8	0 4
	133	100 0	5 5

Control group

The control group consisted of 133 children with normal screening results but referred to the phonia trist because of speech defects They went through a professional examination at the Department of Audiology generally several months up to more than one year after the screening

RESULTS

Out of the 2 573 children living in Lund and Dalby 2 447 or 95 1 % participated in the study Seven children were under current pro fessional care because of ear diseases and were not screened Of the remaining 2 440 children screening audiometry could be performed on 2 414 children (1 251 boys and 1 163 girls) or 98 9% According to the criteria 133 children (5 5%) were referred to the audiologist for impaired hearing Another 10 children (5 boys and 5 girls) (0 4%) were referred for symptoms of adenoids without hearing impairment and were treated with adenoidectomy

Nine children out of the 133 did not come to the audiological examination The remaining 124 children were classified according to their main otological diagnosis (Table 1)

Seventy three children had a middle ear affection causing their hearing impairment In half of them (37 children) this affection could be cured and their hearing restored to normal by simple otological measures (Politzer antibiotics nasal decongestants in some cases myringotomy), only few visits to the clinic were required

The other half (36 children) required more intense therapy (adenoidectomy tonsillectomy, drainage tubes) and in general, several visits before the hearing was normalized

An excess of cerumen and detritus caused hearing defects in 16 children After removal which in two boys had to be done under general anaesthesia the hearing was normal

Only 5 cases of sensorineural hearing impair ment were found 2 boys and 3 girls In 3 of these children, the cause of their hearing de fect was thought to be congenital or hereditary, one child had complications from an encepha litis and the remaining child had a noise injury In one girl the hereditary lesion was severe enough for use of a hearing aid to be con sidered She was also slightly retarded in language development The other 4 children received no special treatment

When examined by the audiologist 30 chil dren, or 22 5% of those referred were found to have normal hearing Eight of these chil dren were examined within 2 weeks after the screening the remaining 22 from three weeks up to one year afterwards

There was an even distribution of boys and girls among those referred and also in the different subgroups of otological findings ($p > 0\ 05$)

During the first year when only 3 fre quencies were used at the testing 30 children were referred (4 7%) 7 were normal and 7 did not come to the audiological examination During the following years 103 children were referred (5 8%) 23 were normal and 2 did not come There was no difference in the rate of referral overreferral or pathological find ings of those referred between these two pe riods ($p > 0\ 05$)

Control group

The audiological examination of the 133 chil dren in the control group revealed normal hearing in 128 children Five had a slight conductive hearing loss due to middle ear infections and were cured by simple measures

Table 2 *Information from the questionnaires in relation to actual findings of hearing impairment*

Questionnaire	Middle ear infection n = 73		Sensori neural hearing loss (n = 5)	Cerumen (n = 16)	Normal findings n = 30		Not referred n = 2 281[a]		Total n = 2 405	
Hearing loss among parents or siblings	0	0	1	0	0	0	16	0 7	17	0 7
Hearing loss among other relatives	2	2 7	0	0	1	3 4	104	4 6	107	4 4
Previous otitis media	31	42 5	1	3	9	30 0	675	29 6	719	29 9
Actual history of adenoids (snoring open mouth thick speech)	26	35 6	2	5	6	20 0	404	17 7	443	18 4
Staying at day nurseries or kindergartens	18	24 7	0	4	4	13 8	329	14 4	355	14 8
Delayed language development										
Speaking single words after the age of 15 months	12	16 4	0	1	7	23 3	287	12 6	307	12 7
Speaking sentences after the age of 36 months	9	12 3	0	1	4	13 3	133	5 0	127	5 3
Present complaints of impaired hearing	12	16 4	0	3	1	3 3	59	2 6	75	3 1

[a] Nine children who did not come to the audiologist are excluded

Questionnaire

The results from the questionnaires relevant to the auditory examination are presented in Table 2

Hearing loss in the families was seldom reported only in 0 7 There was no difference in this respect between children with normal and pathological screening audiogram ($p >$ 0 05) Previous otitis media was reported in 42 5% of the children with current middle ear infections as the cause of hearing impairment but in significantly fewer children 29 5 / ($p < 0\ 05$) without signs of middle ear infections The infected children also had symptoms of adenoids to a greater extent ($p < 0\ 001$) and their parents had noticed actual impaired hearing in 16 4 compared to 2 7% in non infected children ($p < 0\ 001$) It was stated that 12 3 of the infected children and 5 1 / of the non infected children began speaking sentences first after the age of 36 months ($p <$ 0 05)

DISCUSSION

The present report describes the possibilities of including a screening for hearing defects in a general health control of 4-year-old children performed at an ordinary health center by ordinary nurses although specially trained

The screening with a pure tone audiometer according to the play audiometry principle was easy to learn for the nurses and the children cooperated very well Only in 26 children 1 1% could the testing not be carried out The disadvantages of using an ordinary room instead of a sound proof box when testing made it necessary to raise the level of acceptance from 20 to 25 dB at 250 cps An advantage of this arrangement was that the children were more familiar with the surroundings

The rate of reference from the screening 5 5 is well in accordance with some other Swedish investigations (2 9) The higher frequencies of hearing loss found in smaller studies 8–10% (6) 10 (4) and 25 (12)

Table 3 *Evaluation of the effectiveness of pure tone audiometry screening when different combinations of frequencies are used Results from 101 children born 1964–65*

Combination of screening frequencies	Professional diagnosis Mild infection (*n*)	Severe infection (*n*)	Cerumen (*n*)	Sensori neural (*n*)	Normal (*n*)	Total no of children (*n*)
I 250 1 000 4 000 cps	20	23	11	1	8	63
II 250 4 000 8 000 cps	30	30	14	2	23	99
III 250 1 000 4 000 8 000 cps	30	30	14	2	23	99
IV 250 500 1 000 2 000 4 000 8 000 cps (Total)	31	31	14	2	23	101

may be explained by biased sampling due to overparticipation of children with suspected hearing loss In Dissevelt s study (4) the pre-school children were taken from kindergartens and it is well known that children in kinder gartens and day nurseries run a greater risk of catching infections than other children A total survey of an unselected population of pre school children has not been published before

As expected the main cause of the hearing losses was conductive impairment (66 9%) middle ear infections in 54 9% and obturating cerumen in 12% In our study children with middle ear infections causing hearing loss, had a slightly retarded language development (re ported age when speaking sentences Table 2) compared to other children $p > 0\ 05$ Previous studies (5 7 8) have also revealed that chronic or recurrent middle ear infections may con stitute a major deprivation of auditory stimuli and therefore be a cause of retardation in language development As most conductive im pairments in children are reversible if treated early it should be wise to include a hearing screening in routine examinations of children

In our material 355 children or 14 8% attended day nurseries or kindergartens These children had significantly more hearing loss due to middle ear infections than others 5 1% vs 2 7% ($p < 0\ 05$) Also they more often had severe middle ear infections than other children 3 1%, vs 1 2% ($p < 0\ 05$) The rate of hearing impairment due to infections is about the same in our kindergarten children, 5 1%, as in Dissevelt s material 8 6% ($p > 0\ 05$)

The findings of sensorineural hearing im pairment, 0 2% of all screened is also in ac cordance with other investigations (2 4 6 9) Only one of our 5 children had a more serious hearing loss Another 2 children were already under professional care and received special education because of serious sensorineural hearing loss Furthermore among the 126 children who did not attend the health control at all 3 were already taken care of in a pre school for deaf children Thus based on this survey of a complete population of 4 year old children it may be estimated that in these areas about 2 per 1 000 4 year old children have a serious sensorineural hearing impair ment requiring special care and training The most severely handicapped children were de tected at the age of 1–2 years These findings are in close agreement with the calculations of WHO (13)

The frequency of overreference in our study was 1 2% or 22 5% of the children referred In a recent similar investigation from Stock holm (2) the rate of overreference was signifi cantly higher 39 4% of children referred ($p < 0\ 02$) The explanation of this difference may be that our children were treated with nasal decongestants and re tested before they were sent to the audiologist In Dissevelt s in vestigation (4) 28 children out of 71 having failed the first screening were normal when re tested after 2 weeks Since many of our

overreferences had a long delay before they were investigated by the audiologist it is reasonable to assume that some of the slight conductive impairments were cured spontaneously during that time If the organization permits it would perhaps be wise to re test several times the children with hearing defects at the screening in order to reduce the burden of healthy children on the audiologist

Another reason for overreference as pointed out by Barr (2) could be that the external auditory ducts were constricted by the pressure of the earphones thus leading to an impaired conduction of sound This phenomenon however was not consistently investigated in our study

The examination of the control group although rather small showed that it is unlikely that many children with severe hearing impairment were missed at the screening test The 5 children with slight conductive hearing loss could very well have developed their middle ear infections between the two examinations (cf the high frequency of reported otitis media in Table 2)

Although the rate of pathological findings was the same when 3 frequencies were tested in children born 1963 as when 6 frequencies were tested on children born 1964–65 it is evident from Table 3 that some children in need of treatment would have been overlooked during the last 2 years had only the same 3 testing frequencies been used as during the first year Our data indicate that screening at 250 4 000 and 8 000 cps seems to be the best combination in detecting hearing impairment if only 3 frequencies are to be used better results are not reached with 4 frequencies As the screening method is easy and quick to carry out it seems wise however to cover the most important range of hearing for speech 250–8 000 cps (7) by testing at 6 frequencies

Although some children seemed to be in the risk zone for developing hearing losses e g children with previous otitis media and with present symptoms of enlarged adenoids or of impaired hearing as judged by the parents this information from the parents was not selective enough to be of practical value Thus in order to detect hearing impairment in pre school children it is necessary to perform a screening examination

SUMMARY

In a general health control of an unselected population of 4 year old children an auditory screening was included Examination with a pure tone audiometer could be performed on 2 414 children or 98 9 % and 5.5% were referred for further evaluation of newly detected hearing impairments Most of the referred children had a conductive hypoacusis (66 9 %) including mild middle ear infections in 27 8 % severe middle ear infections in 27 1 % and cerumen in 12 0 % Five children, 3 8 % of those referred and 0 2 % of all screened had a sensorineural hypoacusis Another 5 children in the total population already received special care and training because of severe hearing loss Thirty children 22 5% of those referred and 1 2 % of all screened had normal hearing when examined by the audiologist

The method of examination was easy to perform and accurate enough for screening purposes It is recommended that the whole range from 250 to 8 000 cps should be covered by testing at 6 frequencies

From a questionnaire to the parents children with previous otitis media present signs of enlarged adenoids or complaints of impaired hearing were found to be in the risk zone for suffering hearing loss This information was however not selective enough to be of practical value as a screening method

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LATE VITAMIN B_{12} DEFICIENCY FOLLOWING RESECTION OF THE ILEUM IN THE NEONATAL PERIOD

H B VALMAN

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Vitamin B_{12} in physiological doses is absorbed exclusively from the distal half of the small intestine in adults (9 4 17) Previous reports (7 11) have described deficiency of vitamin B_{12} in infants who have had a large part of the jejunum as well as the whole of the ileum removed in the neonatal period In these infants a low serum vitamin B_{12} level was discovered within 4 years of the resection However in 2 patients the preservation of only 10 cm (18) and 16 cm (3) of terminal ileum after major neonatal resections of the small intestine was sufficient to allow normal absorption of vitamin B_{12} Similarly Clayton & Cotton (8) noted that vitamin B_{12} absorption was normal in an infant with 38 cm of terminal ileum after a resection at the age of 10 months

In the present study serum vitamin B_{12} levels and vitamin B_{12} absorption have been investigated at puberty in 2 children both of whom had resections of the ileum in the neonatal period Their early progress has been described previously (21 25)

METHODS

Haematological methods were standard (10)

Serum vitamin B_{12} was assayed using *Euglena gracilis* strain Z (1) The normal range is 160–925 pg/ml

Serum folate was measured using *Lactobacillus casei* (23) The normal range by this method is 6–21 ng ml but in patients with megaloblastic anaemia due to folate deficiency the levels are below 3 ng/ml

The red cell folate levels were measured by estimating the *L casei* activity of haemolysates of sequestrened whole blood samples (15) the normal range being 160–640 ng ml of packed red cells

Urinary methylmalonic acid was measured as described by Oberholzer et al (19) and the upper limit of the normal range is 5 mg in 24 hours.

Vitamin B_{12} absorption was determined using an oral dose of 1 μg of ^{58}Co-labelled vitamin B_{12} plus intrinsic factor and the whole body counting technique (2?)

CASE REPORTS

Case 1

J B aged 1? years She was born in 1958 weighing 2 8 kg When she was 13 hours old a resection of the terminal ileum with ileostomy was performed for ileal atresia with a volvulus The fixed specimen was 65 cm in length Two weeks later an end to-side anastomosis was carried out between the distal end of the remaining ileum and the caecum After a short period of intravenous fluids alone she was given expressed human milk and later a half-cream cow s milk mixture

Frequent offensive stools persisted and she was only 850 g above her birth weight when she was discharged at the age of 2 / months She received a low fat and high protein diet with added vitamins A B C D and E Short episodes of rectal bleeding occurred at 5 months 7 months and 8 months of age but sigmoidoscopy and barium enema showed no abnormality From the age of 11 months there was progressive weight gain and she passed one formed stool daily The first serum vitamin B_{12} estimation carried out at the age of 3 years was 140 pg/ml During a Schilling test the usual flushing dose of 1 mg of unlabelled vitamin B_{12} was given but the test was technically unsatisfactory The subsequent serum vitamin B_{12} levels are shown in the figure

At the age of 11 years 5 months when she was on

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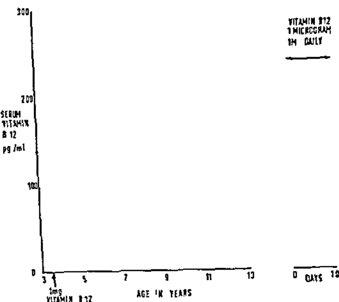


Fig. 1. Serum vitamin B_{12} levels in case 1 (J. B.).

have considered that the handling of the gut necessary for careful measurement was unjustified as a routine procedure in such extremely ill babies often with peritonitis, septicaemia and electrolyte abnormalities.

The marked steatorrhoea with impaired absorption of vitamin B_{12} in Case 1 suggested the possibility of the stagnant loop syndrome. However, the failure to demonstrate an anatomical stagnant loop during barium studies, the absence of pathogens in the duodenal aspirate, the normal urinary indicans excretion and failure of a course of antibiotics to improve the absorption of vitamin B_{12} suggested that the presence of the stagnant loop syndrome was unlikely.

Interval before serum vitamin B_{12} level falls

In adults following total gastrectomy the serum vitamin B_{12} level may not fall for 10 years due to large stores in the liver (5). Even in adults where malabsorption may be virtually complete after ileal resection as long as 6 years have elapsed before the serum vitamin B_{12} level has fallen (5). In Case 1 the serum vitamin B_{12} level fell abruptly from 168 pg/ml to 72 pg/ml over a period of 6 months during puberty. Since the serum level had been maintained just within normal limits for 2 years (Fig. 1) it seems probable that the pubertal growth spurt precipitated the fall. Helge (14) described a 13 year-old girl with megaloblastic anaemia due to vitamin B_{12} deficiency 5 years after resection of 80 cm of the terminal ileum. The pubertal stage of that patient was not mentioned.

The only supplementary dose of vitamin B_{12} J. B. (case 1) had received was 1 mg intramuscularly during the Schilling test at the age of 3 1/ years and about 150 μg of this dose would have been retained (6). Presumably the stores present at birth with this additional small dose were sufficient to last 12 years.

SUMMARY

Vitamin B_{12} deficiency developed in a child at puberty after resection of 65 cm of the terminal ileum in the neonatal period and this was associated with demonstrable malabsorption of vitamin B_{12}. In contrast another child who had had a similar length of the ileum removed but with preservation of the terminal 12 cm maintained a normal serum vitamin B_{12} level and absorbed vitamin B_{12} normally. Therefore infants who have had a resection of

Table 1 *Results of clinical investigations*

	Case 1 (J B)	Case 2 (A D)	Normal
Haemoglobin g/100 ml	14 5	13 3	12–14 5
Stained blood film	Normal	Normal	
Serum vitamin B_{12} pg/ml	72	624	160–925
Serum folate ng/ml	13 6	3 8	6–21
Red cell folate ng/ml	—	153	160–640
Serum iron μg/100 ml	65	133	80–150
Iron binding capacity μg/100 ml	294	347	350–410
Plasma calcium mg/100 ml	10 1	10 0	9 5–11 5
Plasma phosphorus mg/100 ml	4 2	4 4	3 5–5 5
Plasma alkaline phosphatase μg/min/l	125	139	90–180
Plasma cholesterol mg/100 ml	124	—	100–220
Serum albumin g/100 ml	5 0	—	4 8–5 6
Vitamin B_{12} absorption	4	72	40 *
Vitamin B_{12} absorption after ampicillin *	8	—	40

a low fat high protein diet with no added vitamins her height was 134 7 cm (10th percentile) and her weight 29 0 kg (10th percentile) There was no abdominal distension Early changes of puberty were present with sparse pubic hair and early breast development There were no abnormal neurological signs

Case 2

A D aged 12 years His birth weight was 2 5 kg An ileal resection was carried out when he was one day old for a volvulus associated with malrotation of the gut the remaining terminal ileum was 15 cm The fixed specimen was 67 cm in length Oral feeds were begun with expressed human milk but there was persistent diarrhoea and poor weight gain However when he was 7 weeks old the diarrhoea became less severe and a half cream cows milk mixture was gradually introduced He was discharged home at 11 weeks weighing 1 2 kg above his birth weight

He had episodes of frequent loose stools until the age of 3 years but thereafter his bowels were open once a day and the stools were brown and solid

At the age of 12 years when he was receiving a normal diet with no added vitamins his height was 148 0 cm (above the 50th percentile) and his weight was 39 5 kg (above the 50th percentile) There was no abdominal distension Pubic hair was present His voice had started to become deeper in character He had never received supplementary vitamin B

INVESTIGATIONS

The results of investigations are listed in Table 1 Other findings in Case 1 are summarised as follows faecal fat excretion 19 5 g daily (3 day collection on daily intake of 70 g) Radiograph of wrist and hand showed a bone age compatible with the chronological age and there was no evidence of rickets Barium follow through revealed that the stoma at the site of the anastomosis between the ileum and the caecum was functioning normally and there was no anatomical evidence of a stagnant loop in the small or large intestine Aerobic and anaerobic culture of duodenal aspirate grew no pathogens The urinary excretion of indicans was 64 mg in 24 hours On her normal diet the 24 hour urinary excretion of methyl malonic acid was 12 5 mg and after 5 g L valine it was 12 3 mg

DISCUSSION

Length of resection

Booth (5) found that in adults vitamin B_{12} absorption was normal when less than 60 cm of ileum were removed but was usually impaired when 180 cm or more were removed Fone et al (13) concluded that vitamin B_{12} absorption was normal after resection of less than 30 cm and usually impaired when a greater length was removed although it was normal in one patient who had lost as much as 120 cm In most of these patients resection was performed for regional enteritis and probably some of the remaining bowel was abnormal

The results in Case 1 also show that following neonatal resection of the ileum the remaining small gut does not absorb adequate amounts of vitamin B_{12} The length of our patients resections would correspond to about 130 cm in the adult since the length of the small gut in the adult is about twice that in the newborn infant (2 12 20) The results of the tests in Case 2 show that as little as 12 cm of the terminal part of the ileum may be sufficient for normal vitamin B_{12} absorption and impaired absorption cannot be predicted solely from the length of intestine resected Measurement of the length of the remaining small intestine might be a better indication of absorptive capacity (24) However some surgeons

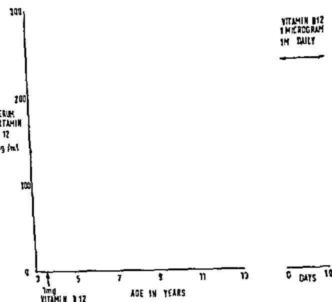


Fig 1 Serum vitamin B levels in case 1 (J B)

have considered that the handling of the gut necessary for careful measurement was unjustified as a routine procedure in such extremely ill babies often with peritonitis septicaemia and electrolyte abnormalities

The marked steatorrhoea with impaired absorption of vitamin B_1 in Case 1 suggested the possibility of the stagnant loop syndrome However the failure to demonstrate an anatomical stagnant loop during barium studies the absence of pathogens in the duodenal aspirate the normal urinary indicans excretion and failure of a course of antibiotics to improve the absorption of vitamin B_1 suggested that the presence of the stagnant loop syndrome was unlikely

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INFANT FEEDING HYGIENE IN SWEDEN

A Survey of Bottle and Teat Hygiene

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In bottle feeding of infants hygiene is of out most importance if dangerous gastrointestinal infections are to be avoided Traditionally the mothers are informed about using only clean utensils boil the water used for dilution of the feeds boil the milk if it is not purchased in a sterile form and also boil the bottles and teats frequently In the US in addition it has been advocated to perform a terminal steriliza tion of the prepared formula in the bottle The increasing use of ready to feed formula or al most ready where only water has to be added has simplified bottle feeding The boiling of bottles and teats now takes a considerable time for the mothers and this procedure is therefore omitted now and then Simpler meth ods for disinfection of the feeding utensils thus are desirable

In Sweden the infant mortality is low and the morbidity in gastroenteritis relatively low This might depend upon either a good standard of hygiene or might be explained by the cool climate of Sweden Hence an investigation was performed[1] in the city of Sundsvall an in dustrial town on the East coast of Sweden The aim of the investigation was to study the degree of bacterial contamination of feed ing bottles and teats in the home with the methods used by the mothers and repeat the same examinations after introduction of chem ical disinfection of bottles and teats with a solution of sodium hypochlorite (the Milton method)

[1] The investigation was performed thanks to the support of Vick International which company also supplied the Milton solution used

MATERIAL AND METHODS

Sampling

Over a period of 9 days in May 1971 6 nurses from the Sundsvalls Well Baby Clinics accompanied by 2 field investigators of Vick International forming 2 groups per day visited in all districts of Sundsvall more than 150 homes where there was a young baby and invited the mothers to cooperate in this study If the mother agreed and the baby was fed with bottles at least twice a day and there was available on this first visit a feeding bottle and a teat which the mother considered sterile" and/or ready for filling, these were collected by the field investigators with aseptic precautions having the mother place the bottle and the teat in a sterile plastic bag

The collected bottles were delivered to the city laboratory for bacteriological assessment

The selection of homes visited was left to the nurses of the Well Baby Clinics and included practically all mothers with bottle fed children up to 6 months registered in their districts

Since the nurses call regularly at these mothers to look after the baby—sometimes also on the mother s request—the mothers had no prior warning regarding the purpose of the visit. Hence bottles and teats had received only their usual cleansing and decontamina tion treatment Moreover the visits were made every day in a different neighbourhood

After completion of a questionnaire and collection of bottle and teat the field investigator informed the mother about the chemical disinfection method with Milton (hypochlorite solution) and invited them to use the method for a period of about 3 weeks instead of any other method for bottle and teat pro cessing practiced so far

After a practical demonstration of the Milton method a new bottle and teat in replacement of the

the ileum should be tested for vitamin B_{12} absorption If absorption is impaired, the serum level should be regularly assayed at least until adult life so that treatment may be given before complications arise

ACKNOWLEDGEMENTS

I wish to thank Dr P D Roberts for facilities for carrying out the vitamin B absorption tests in the Department of Haematology at the West Middlesex Hospital I am indebted to Dr B Levin and Dr V G Oberholzer for the haematological and biochemical estimations and Dr A V Hoffbrand for the microbiological assays

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Key words Vitamin B_{12} deficiency ileal resection neonatal period

pants as 8 families had left for vacation or moved. Of the remaining 139 twelve had to be excluded on different accounts. One mother had misunderstood how to use the Milton solution, 3 babies refused the teat because of the chlorine smell, three mothers feared allergy to chlorine and five mothers considered it unnecessary to bother so much about the care of feeding utensils. Thus 127 mothers took part in the second sampling of bottles and teats.

In Table 1 is listed the age of the infants at the start of the study. 73 of the infants had been weaned from the breast before the age of 4 weeks, another 54 before 8 weeks. All infants were fed ready made formulas in powdered form which are prepared through diluting with water only. As is seen in Table 2 most mothers prepare the formula immediately prior to feeding. Hence the number of bottles and teats in use at the same time is kept low (Table 3).

At the interview 110 mothers said they were boiling the bottles, 127 boiled the teats, the rest used only hot water for cleaning the bottles and teats. The mothers were asked in addition for how long time they used to boil the bottles and teats. The answers are given in Table 4. How frequently the bottles and teats are boiled is seen in Table 5. This table shows that only about half of the bottles and teats are boiled every day.

At the visits the nurses also checked the methods for storing the bottles and teats. Most were kept in cupboards or drawers, but a considerable number in the open (cf. Table 6). According to the mothers 65 of the 147 infants at least at one occasion had suffered from vomiting and diarrhoea.

After the introduction of the chemical method for disinfection of the feeding utensils most bottles and teats were stored in the hypochlorite solution (Table 7). The hypochlorite solution is at least partly inactivated in the presence of protein. Thus it is important to clean the bottles and teats thoroughly before they are immersed in the Milton solution. The teats should be rubbed with salt to remove the milk left, but 20 of the mothers (i.e. 15 %) considered this inconvenient and never did it. Another 15 of the mothers did it occasionally, whereas 92 or 74% of the mothers rubbed regularly with salt.

Table 5. *Boiling frequency of bottles and teats*

	Bottles	Teats
Prior to each meal	5	5
Not always prior to each meal	3	3
Twice a day	5	5
Once a day	39	54
Every other day	15	16
Twice a week	19	15
Once a week	15	17
Less often	9	12
Total	110	127

Table 6. *Storage of bottles and teats which were boiled or cleaned only*

		Storage			
	Total	Covered up	Not covered up	In a cupboard or drawer	In refrigerator
Bottles	147	13	49	78	7
Teats	147	47	50	49	1

Table 7. *Storage of bottles and teats between meals*

Storage place	Bottles		Teats	
In the Milton Box	125	99	122	96
Covered up outside M B	0		2	1
Not covered up	2	1	3	2
Total	127		127	

Table 8. *Opinion of mothers regarding the acceptability (as expressed by convenience and practicability) of the Milton method compared to boiling*

Classification	No of mothers	%
Accepted better than boiling	93	74
About as well accepted as boiling	23	18
Not as well accepted as boiling	7	5
No opinion	4	3
Total	127	

Table 1 *Age of children*

Age	No
Less than 3 months	24
3 to 4 months	64
5 to 6 months	39
More than 6 months[a]	20
Total	147

[a] These children were bottle fed at least twice a day

Table 2 *Frequency of filling bottles*

Filling times/day	No
Once/day	8
2 to 3 times/day	8
Immediately prior to each meal	131
Total	147

collected a Milton household box and samples of Milton solution sufficient for 3 weeks were given to the mother

Three weeks after the Milton introduction the nurses and field investigators visited all mothers participating in the study within a further period of 8 days They again collected a bottle and teat which the mother regarded ready for filling (i e after at least 90 min storage in Milton) The bottle and teat collected were placed by the mothers in sterile plastic bags and sent to the laboratory

The following instructions were given to the mothers

Pre cleaning Bottles and teats have to be thoroughly rinsed with cold water using a brush and detergents The teat in addition is to be rubbed inside and outside with table salt and rinsed again under cold water (squeezing water also through orifice to remove milk or salt remnants)

Preparation of the solution To one litre of cold water 12 5 ml of Milton original solution is added (i e filling the special Milton box up to the red mark adding 25 ml) achieving a 1 80 dilution Every 24 hours a fresh solution is to be prepared

The clean bottles and teats are immersed in the solution (removing air bubbles) for at least 90 min Bottles and teats are stored in the solution until use and dripped off only (no after rinse)

Bacteriological procedures

At the laboratory 5 ml of 0 5 % sterile sodium thiosulphate solution was added aseptically to each bottle and shaken thoroughly After standing for 5-10 min one sample of 1 ml was plated onto nutrient agar (Oxoid blood agar base No 2) and incubated for 24 hours at 36 C another sample of 1 ml was used to inoculate 5 ml of MacConkey broth (Oxoid CM 6) which was then incubated at 44 C for 24 hours

Teats were sampled by running 2 ml a 0 5% sterile solution of thiosulphate into the inverted teat After draining through the teat orifice for 5-10 min any remaining rinse in the teat was forced through the orifice or if this proved impossible the rinse was sampled in situ One ml of rinse was used to inoculate a plate and the remainder of the rinse was added to 5 ml of broth Incubation temperature and time was as for the bottle samples

Plates with 0-500 colonies/plate were accurately counted those with 500-1 000 were counted to the nearest 20 and plates with over 1 000 were placed in two categories by density of growth as 1 000+ or 10 000+

The results of the bacteriological examinations were graded as follows (4)

0-10 colonies/plate with negative coliform test = A good

10-100 colonies/plate with negative coliform test = B fair

100 or more colonies/plate and/or positive coliform test = C poor

RESULTS

152 mothers were invited to take part in the study, of these 5 were not interested as their children were soon to be weaned from the bottle Thus 147 mothers took part in the study In the second part of the investigation the nurses called on the mothers 175 times but could reach only 139 of the 147 partici

Table 3 *Number of bottles and teats used*

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3 to 5 min	51	51
6 to 10 min	10	13
More than 10 min	7	5
No opinion	4	3
Total	110	127

little knowledge of bacteriology consider the boiling of teats and feeding bottles for 10 min as unnecessary or at least exaggerated In addition the teats are rather rapidly destroyed when boiled frequently glass bottles break easily if they are cooled too rapidly or modern plastic bottles melt or lose their shape if they are in contact with the bottom or sides of the hot pan during sterilization This may at least partly explain the findings in Table 4 which shows that the boiling time for bottles and teats is too short

The storing of the bottles and teats was not satisfactory in most cases but this does not influence the end results The bottles and teats stored in the open did not show any significantly worse bacteriological results than those stored under cover

We have not been able to show in this study that exactly those infants who had been ill with diarrhoea or vomiting also had the most heavily contaminated teats or bottles But from the Tables 5 and 9 might be concluded that with the methods used for disinfection in the home dangerous contamination might occur Boiling correctly done of course is very effective but for use in the home chemical methods of disinfection appear to be more reliable than boiling (1 2 3) In Sundsvall as in Reading and Hamburg where similar studies were made (3 4) a chemical method of disinfection of bottles and teats lead to significant improvement in hygiene The chemical method is rather simple and was preferred by a majority of mothers (74°) only 5 ∘ preferred boiling and 18% considered one method as simple as the other (Table 8)

Table 12 *Comparison between levels of contamination of bottles and teats after processing methods other than Milton that is boiling and rinsing with boiling water in Hamburg Reading and Sundsvall*

Gradings (Col /ml)	Hamburg ( )	Reading ( )	Sundsvall ( )
Bottles			
A = good B fair +	21	51	44
C = poor	79	49	56
Teats			
A = good B = fair +	30	27	30
C poor	70	73	70

Table 13 *Comparison between levels of contamination of bottles and teats after processing with Milton and Reading and Sundsvall*

Gradings (Col/ml)	Reading ( )	Sundsvall (*)
Bottles		
A = good B = fair +	86	97
C = poor	14	3
Teats		
A = good B = fair +	65	79
C = poor	35	21

[a] Significantly better than Reading (p ~ 1)

SUMMARY

A study was performed of the bacteriological contamination of feeding bottles and teats in the home A comparison between the method used by the mothers (rinsing in hot water or boiling for a short time) and the use of dilute sodium hypochlorite showed a significant improvement with the chemical disinfectant

ACKNOWLEDGEMENT

The bacteriological investigation of bottles and teats was carried out by G D Breach and M Bonneywell at the City laboratory Halsovårdsnamnden Sundsvall through the courtesy of Vet Dr T Bjorkman

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Table 9 *Overall bacteriological results from both samplings (I and II) bottles and teats Colony counts and E coli tests per gradings*

Colonies/ml and coliforms gradings	Bottles after processing methods other than Milton (boiling etc)		Bottles after processing with Milton		Teats after processing methods other than Milton (boiling etc)		Teats after processing with Milton	
A = good	56	44	123	97	39	31	100	79
B = fair	30	24	1	0 7	24	19	4	3
C = poor	41	32	3	2 3	64	50	23	18
Total	127	100	127	100	127	100	127	100
Coliform test positive	22	17	3	2 3	42	33	12	9

Significant differences between sampling I and II ($p = 1$)

As is seen in Table 8 most mothers thought *the chemical method for disinfection was simp*ler to use than boiling

In Table 9 is shown the bacteriological findings from the two samplings In Table 10 the change in bacteriologic grade between the two methods are tabulated In very few instances the result was inferior with the chemical method in some cases unchanged in most instances a considerable improvement was achieved The number of sterile bottles and teats is shown in Table 11

Similar studies have been performed earlier in Germany and in England A comparison *of our results with the findings in Hamburg* and Reading is given in Tables 12 and 13 The groups B and C in Sundsvall are combined for the sake of comparison As is seen in the tables the differences are only slight

Table 10 *Change in gradings improved unchanged or worse bacteriological results in the 2nd sampling compared to gradings in the 1st sampling*

Change in gradings	Bottles		Teats	
Improved				
B to A	29		22	
C to A	39		46	
C to B	1		3	
Total improved	69	54	71	56
Unchanged				
A	55		32	
B	0		0	
C	1		15	
Total unchanged	56	44	47	37
Worse				
A to B	0		1	
B to C	1		2	
A to C	1		6	
Total worse	2	2	9	7
Absolute total	127	100	127	100

CONCLUSIONS

In Sweden early weaning is becoming more and more popular among the mothers Most infants now get a ready made formula on milk base with vitamins and iron added In this study every infant was fed on these formulas Most mothers prepare each meal immediately before it is consumed thereby reducing th risk of bacterial growth in the food (Table 2) In contrast to the simple way of preparing a meal the rinsing and disinfection of bottles and teats is cumbersome and time consuming It is easy to understand that lay people with

Table 11 *Number of sterile bottles and teats after boiling (and rinsing with boiling water) and after processing with Milton*

Condition	Boiling		Milton	
	Bottles	Teats	Bottles	Teats
Number of sterile	13	11	73	42
Total	127	127	127	127

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BILE ACIDS AND PANCREATIC ENZYMES DURING ABSORPTION IN THE NEWBORN

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Many investigations have shown that the fat absorption coefficient is low in the newborn i e 80–90% (11 27 32) It gradually rises during the first year of life (8 13 31) is higher than 90% in infants older than 9–10 months Provided that the intestinal mucosa functions normally in the newborn the impaired absorption of fat might be due to a low lipase activity or low concentration of bile acids in the intestinal content The concentrations of lipase and bile acids in the intestine during a meal have so far not been investigated in the newborn

Studies on autopsy material have revealed that the concentration of bile acids in the bile is lower in the newborn than in children and adults (2) Investigations of intestinal content carried out after choleretic stimulation with magnesium sulfate confirmed these findings (6) Without previous stimulation the concentration of bile acids was found to be the same as that in children and adults (7 24)

The purpose of the present investigation was to investigate in the newborn the concentration of bile acids and bilirubin in the intestinal content during and after a test meal consisting of breast milk to which polyethyleneglycol 4000 (PEG) was added as a marker enabling to assess the rate of the passage of the breast milk meal For purposes of comparison the concentrations of the pancreatic enzymes trypsin chymotrypsin lipase and amylase were estimated

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CASE MATERIAL

Eight healthy newborn infants 5 boys and 3 girls conforming to the gestational age were examined 3–15 days after birth Gestational age sex birth weight birth length obstetric history and laboratory findings in the infants are given in Table 1

Test meal and sampling

The test meal consisted of pooled breast milk heated to +80 C for 2 min and frozen (−20 C) in aliquots Before the test meal was given to the infants, polyethyleneglycol 4000 (PEG) was added in a concentration corresponding to 5 g per litre breast milk The amount of the test meal equalled that of the ordinary feeds given to each infant i e 40–90 ml The infant was given the test meal by bottle The feeding time did not exceed 20 min in each case

For duodenal intubation on lumen tubes (Argyle® French 5 and 8 91 and 106 cm long respectively) were used They were modified in the following way The tips of the tubes were cut off and a piece of 2 cm of a wider tube (French 10) containing two-three glass pearls were sewn to the cut ends of the tubes Furthermore 3–4 additional perforations were made in the distal 3–4 cm of the tubes

The intubation was performed via the nose (5 infants) or the mouth (3 infants) in conjunction with a regular feed When the tube was felt to have reached the ventricle the pH of the stomach fluid was checked and was found to be lower than 4 in each case The collection of intestinal fluid was performed by siphonage and was started when bile stained contents with a pH higher than 5 appeared in the tube In two cases (L and E) the correct position of the tube i e

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Table 1 *Sex, gestational age, birth weight, birth length, obstetric history, age and laboratory findings in the 8 infants*

Infant	Sex	Gestational age (weeks)	Birth weight (g)	Birth length (cm)	Delivery	Findings at duodenal intubation: Age (days)	Total serum bilirubin (mg/100 ml)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids[a]: C+CD (μmol/24 hrs)	C+CD (μmol/mg creatinine)
W	♀	41	3 660	51	Normal	3	2 8	0 47	0 020
H	o	40	4 080	54	Normal	4	n d [b]	<0 01	<0 001
B	♀	39	3 750	52	Normal	5	10 0	0 13	0 0[illegible]4
N	♂	42	3 650	52	Caesarean section	6	4 0	0 54	0 012
L	♂	40	3 860	51	Forceps	7	3 4	0 39	0 010
Å	♂	40	3 500	50	Normal	10	12 4	0 59	0 021
E	♀	36	3 300	49	Normal	14	2 0	n d [b]	—
P	♂	40	3 850	53	Normal	15	3 4	2 36	0 064

[a] Urine was collected during the 24 hours preceeding duodenal intubation
[b] Not determined

that it was in the horizontal part of the duodenum was also checked by X ray examination The distance between nose or mouth and tip of the tube was 37–66 cm (average distance 54 cm) Fasting samples were collected 15–45 min before giving the test meal which was given at the earliest 4 hours after the previous feeding The intestinal fluid was collected in ice cooled tubes in six successive 5 or 10-minute fractions for 30 min followed by 15–20 minute fractions for altogether 60–240 min Immediately after sampling the intstinal juice was frozen (−20 C)

METHODS

Polyethylene glycol (PEG) was estimated according to Boulter & McMichael (4) The analysis of enzymes was performed on thawed untreated intestinal juice Trypsin and chymotrypsin were analysed according to Haverbach et al (10) Lipase was analysed according to Rick (25) and amylase according to Lagerlof (17) The amylase isoenzymes were separated by acrylamide gel electrophoresis (30) and developed according to Kamaryt & Laxova (15)

The serum concentration of bilirubin was estimated according to Michaelsson (19) The concentration of bilirubin in the intestinal contents was estimated after extraction with ethanol according to Sjovall (28) The same extract of ethanol was used for analyses of bile acids The latter were separated by thin layer chromatography (TLC) before and after solvolysis and hydrolysis as described elsewhere (22) The free bile acids were quantitated by gas-liquid chromatography (GLC) according to Sandberg et al (26)

The urinary excretion of cholic and chenodeoxycholic acids respectively was estimated by GLC as described in another investigation (22)

Calculations
Statistical analyses were performed with Students *t* test

RESULTS

The results of the various analyses are presented in Fig 1 and Tables 1 2 and 3 The PEG concentrations indicate roughly the rate of passage and the degree of dilution of the test meal Rapid emptying of the stomach was indicated in all infants except cases H N, and A PEG was identified in all specimens during 4 hours The concentration of PEG increased initially and thereafter fell rapidly in all except 3 infants (cases H N, A)

Pancreatic enzymes The enzymes trypsin, chymotrypsin, lipase and amylase were determined in all samples Amylase concentrations were invariably very low Several samples from each patient were analysed for amylase isoenzymes Only amylase showing the same electrophoretical mobility as saliva amylase was identified

The concentrations of trypsin chymotrypsin and lipase in the fasting samples varied greatly from one infant to the other As soon as the test meal had reached the intestine a decrease of the enzyme concentration occurred in all

Table 2 *The concentrations of trypsin and chymotrypsin in the intestinal contents*

Infant	Fasting values			Concentrations during absorption (mean ± S D)			Correlation coefficient
	Trypsin (μg/ml)	Chymotrypsin (μg/ml)	Ratio Trypsin/ Chymotrypsin	Trypsin (μg/ml)	Chymotrypsin (μg/ml)	Ratio Trypsin/ Chymotrypsin	
W	8	44	1 9	65 7 ± 46 9	32 2 ± 19 6	1 8 ± 0 5	0 983
H	175	168	1 0	59 6 ± 35 8	80 3 ± 29 7	0 7 ± 0 1	0 984
B	218	114	1 9	176 1 ± 136 7	126 2 ± 102.9	1 4 ± 0 2	0 945
N	57	27	2 0	117 9 ± 70 2	99 9 ± 51 7	1 2 ± 0 2	0 932
L	57	60	1 0	71.3 ± 59 9	76 0 ± 62 1	0 9 ± 0 1	0 990
Å	234	224	1 1	150 9 ± 75 0	170 9 ± 73 1	0 9 ± 0 1	0 975
E	37	78	0 5	36 9 ± 22 3	53 4 ± 39 4	0 9 ± 0 4	0 914
P	101	87	1 2	72 2 ± 57 5	53 0 ± 43 8	1 4 ± 0 1	0 994

infants except infants H and N Thereafter a slow increase of the enzyme concentrations occurred which in 2 infarts did not exceed the fasting values The concentrations of trypsin chymotrypsin and lipase were well correlated in each infant

The ratio trypsin/chymotrypsin ranged from 0 5 to 2 0 (Table 2) In each infant this ratio varied slightly during the test meal as was shown by the correlation coefficients (Table 2) The ratio trypsin/lipase varied greatly from one infant to the other In 2 infants cases W and H the concentration of lipase was markedly lower than that of trypsin In no infant did any appreciable change in the ratio trypsin/lipase occur during the test meal

Bile

Bilirubin The fasting values varied greatly from infant to infant In all except 1 infant (Case N) a decrease occurred when the test meal had reached the intestine Thereupon the concentration of bilirubin slowly increased to values which were within the same range of variation as observed in fasting samples

Bile acids TLC analyses of each sample of intestinal contents showed only the presence of conjugated bile acids mainly glycine and taurine conjugates Unconjugated bile acids were absent After solvolysis and hydrolysis TLC and GLC disclosed the presence of mainly cholic and chenodeoxycholic acids Deoxycholic acid was absent In all infants trace amounts of bile acids with the same TLC mobilities and GLC retention times as mono hydroxycholanoic acids were identified The ratio cholic/chenodeoxycholic acid varied from 0 9 to 3 0 (Table 3)

The total concentration of cholic and chenodeoxycholic acids respectively was determined (Fig 1 and Table 3) The concentrations showed great variations in fasting samples and decreased in all except 2 infants when the test meal had reached the intestine Thereafter they increased to values corresponding to those observed during the fasting period Only in infants B and N did the concentration of bile acids rapidly increase suggesting contraction of the gallbladder The mean concentration of

Table 3 *The concentrations of cholic (C) and chenodeoxycholic (CD) acids in the intestinal contents*

Infants	Fasting value		Concentrations during absorption (mean ± S D)	
	C + CD (mmol/l)	Ratio C/CD	C + CD (mmol/l)	Ratio C/CD
W	1 83	1 7	1 31 ± 0 98	1 5 ± 0 5
M	5 39	1 5	0 56 ± 0 36	1 1 ± 0 2
B	1 53	0 9	1 17 ± 1 60	1 1 ± 0 1
N	0 65	1 0	1 43 ± 1 73	1 4 ± 0.2
L	0 50	2 2	0 41 ± 0 32	2 5 ± 0 5
Å	3 96	3 0	0 76 ± 0 67	1 9 ± 0 6
E	0 85	1 7	1 08 ± 0 66	1 3 ± 0 2
P	1 99	1 5	1 48 ± 1 38	2 2 ± 0 6

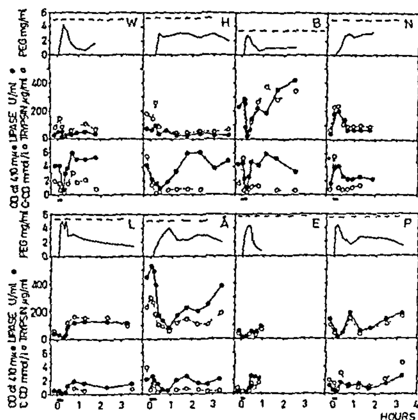


Fig 1 The concentrations of poly ethyleneglycol (PEG) lipase trypsin bilirubin (OD) and cholic (*C*) and chenodeoxycholic (*CD*) acids in the intestinal content before and during a breast milk meal in 8 infants Capital letters mark the individual infant concentration of PEG in the test meal — feeding time

cholic- and chenodeoxycholic acids combined varied during the test meal ranging from 0 41 to 1 48 mmol (Table 3)

DISCUSSION

In the present investigation the pancreatic enzymes and bilirubin and bile acids were determined simultaneously in the duodenal contents after a test meal of pooled breast milk The concentrations are influenced by the emptying of the stomach and the excretion of pancreatic fluid and excretion of bile In 5 infants rapid emptying of the stomach occurred This observation is in agreement with those made by earlier workers (5) Our results have shown that the concentrations of both the pancreatic enzymes and bile constituents after the test meal decreased initially This may have been due to excessive dilution of the intestinal contents during the rapid emptying of the stomach

As in earlier studies (9 14 16) a low concentration of amylase which mainly consisted of salive amylase was observed in this investigation The concentration of trypsin chymotrypsin and lipase respectively, varied greatly from one infant to the other Similar observations were made by Hadorn et al (9) and Zoppi et al (33) in their studies of pancreatic excretion after pancreozymin secretin stimulation

In adults the total bile acid concentration varies ranging from 2 5 to 10 mmol during a test meal (20 29) It is difficult to define the critical micellar concentration (CMC) of bile acids in intestinal contents as it is influenced by the ratio dihydroxy/trihydroxycholanoic acids the ratio glycine/taurine conjugates as well as by other compounds than bile acids (12) In adults with non alchoholic liver cirrhosis (1) or viral hepatitis (21) incomplete fat absorption invariably occurs if the mean total bile acid concentration is lower than 4 mmol In the present investigation the concentrations of dihydroxy and trihydroxy cholanoic acids in the intestinal contents was consistently very low The mean concentration during the test meal varied in the 8 infants being 0 41–1 48 mmol (range of variation being 0 02–5 29 mmol) Thus the concentration

of bile acids in the intestinal contents is probably too low to permit optimal fat digestion in the newborn

The low concentration of bile acids in the intestinal contents might mainly have been due to the following three factors incomplete emptying of the gallbladder deficient concentration of gallbladder bile or low concentration of bile acids in the hepatic bile Only in 2 infants did the excretion of bile acids increase initially The possibility of a gallbladder contraction can therefore not be ruled out However the rise of the concentration of bile acids was slight compared with that occurring in adults in conjunction with emptying of the gallbladder (29) Other investigators have shown that the concentration of bile acids in gallbladder bile is much lower in the newborn than in adults (2 6) Low birth weight infants investigated at 10–19 days of age have been shown to have low intestinal bile acid concentration 2 07±1 3 mmol/l (18) The bile acid concentration increased with age Encrantz & Sjovall (7) have found that the range of variation of the concentration of bile acids in fasting samples was the same as that in adults Our observations were in agreement with their findings as the concentrations of bile acids in fasting samples were often found to be higher than most values during the test meal

The bilirubin concentration in the intestinal contents during the test meal was lower in the infants than in adults (3) The increase in the serum bilirubin concentration supports the assumption that the capacity of the liver for excreting bilirubin is reduced in the newborn None of the infants appeared to have cholestasis because no increase of the conjugated bilirubin was noted and the amounts of bile acids found in the urine were much smaller than those coexistent with cholestasis (22 23)

The present investigation has shown that the concentration of bile acids in the intestinal content of the newborn during a breast milk meal is invariably low and that small amounts of pancreatic enzymes including lipase occasionally are present The low concentration of bile acids found in this investigation may alone be responsible for the incomplete fat absorption in the neonatal period observed by earlier workers (11 27 31 32)

SUMMARY

Eight healthy infants aged 3–15 days were given a test meal of 40–90 ml breast milk after addition of polyethyleneglycol (PEG) Fractional specimens of duodenal contents were collected by siphonage during 1–4 hours using a duodenal tube The samples were analysed for PEG enzyme activity (trypsin chymotrypsin lipase and amylase) bilirubin and bile acids

Amylase was present only in small amounts The concentrations of trypsin chymotrypsin and lipase varied from one infant to the other but were well correlated in each infant

During the test meal the concentrations of bilirubin and bile acids were very low The bile acids mainly consisted of the taurine and glycine conjugates of cholic and chenodeoxycholic acids The total concentrations of cholic and chenodeoxycholic acids combined during the test meal ranged from 0 41 to 1 48 mmol The low concentration of bile acids as an important factor in the reduced fat absorption in the neonatal period is discussed

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PLASMA 17-OHCS LEVELS AFTER VACCINATION AGAINST SMALL POX AND MEASLES IN CHILDREN

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Clinical histopathological as well as laboratory studies point to the fact that the adrenals are stimulated during the course of most infections (1 7 8 9 11 13 14) Since vaccination procedures involve the administration of bacterial or viral products and the production of clinical manifestations which although mild are characteristic of the disease vaccinated against we thought it would be interesting to study the response of the pituitary adrenal system to such procedures We intended to study the adrenocortical response to immunization against small pox and measles

MATERIAL AND METHODS

Two groups of children were studied

I *Children undergoing small pox vaccination* Ten normal healthy infants aged 2 to 3 were vaccinated against small pox All their mothers were vaccinated only once during the first year of life Th vaccine used was that delivered by the Ministry of Health

Pla ma samples for the estimation of 17 OHCS were taken before vaccination and on the fifth and tenth days thereafter

All cases showed the classical local primary reaction to small pox vaccination with some systemic reaction in the form of rise of temp rature and malai e No complications were encountered

II *Children unde go ng measles vaccination* Eleven normal healthy children with no previous history of measles infection were vaccinated against measles Their ages ranged between 8 months and 6 years Each child was given a subcutaneous injection of 0 5 ml of live attenuated measles vaccine (Institute Merieux Lyon France) They were followed up for two weeks after vaccination The mothers of these children contracted measles during their early childhood None was vaccinated against measles

Blood samples for the estimation of plasma 17 OHCS were taken before vaccination and on the seventh tenth and fourteenth days thereafter The reaction to vaccination was variable Four children had mild rise of temperature upper respiratory catarrh and very mild exanthem for two to three days between the seventh and tenth days Seven cases showed no reaction

All the plasma samples for 17 OHCS estimation were taken under basal conditions (8-9 a.m while the children were fasting and not under any other stress) The modified Porter Silber method by Peterson et al (12) was used in the estimation of plasma 17 OHCS levels in the present work

RESULTS

Levels of 17 OHCS before and on the 5th and 10th days after small pox vaccination for the study group are shown in Table 1 It can be seen that all cases except the first showed an increase in level on the fifth day which ranged from 34 to 54 μg/100 ml The mean level on the fifth day was 53 8 μg/ml with a standard deviation of 14 5 μg/100 ml Comparison between the level before vaccination and the level on the fifth day after vaccination showed by use of the *t* test that the two means were significantly different ($p < 0$ 01) The tenth day level was compared with the basal level and all cases invariably showed an increase ranging

Table 1 *Plasma 17-OHCS levels before and after small pox vaccination (μg/100 ml)*

Subject	Basal level	Level after vaccination 5th day	10th day
1	25	25	50
2	17	—	42
3	8	60	12
4	8	48	28
5	20	66	155[a]
6	20	68	54
7	13	47	—
8	10	50	36
9	18	72	56
10	12	48	80
Mean	15 1	53 8	44 8
S D	5 8	14 5	20 4

[a] This reading is not included in the computation of mean and standard deviation because of its extreme value

from 4 to 68 μg/100 ml The mean level on the tenth day after vaccination was found to be 44 8 μg/100 ml, and the standard deviation 20 4 μg/100 ml the difference being highly significant ($p < 0.01$) Case 5 showed a very marked rise (135 μg/100 ml) and was excluded from the analysis because of this extreme value Comparison between fifth and tenth day levels showed that two cases experienced a rise while in 5 cases there was a decrease with an overall average change of 7 9 μg/100 ml The *t* test showed no significant difference between the mean levels on the fifth and tenth days after vaccination ($p > 0.05$)

Table 2 shows the 17 OHCS levels before and after measles vaccination (on the 7th 10th and 14th day after vaccination) On the 7th day after vaccination there was an increase in the level except in one case (subject 4) The average increase reached 39 1 μg/100 ml with a standard deviation of 22 9 μg/100 ml Using the *t* test, it was found that the difference between the two mean levels was highly significant ($p < 0.01$) On the 10th day after vaccination, 7 cases still showed a higher 17 OHCS level 2 cases showed a slight decrease and one case showed no change from basal level The average change was + 31 9 with a standard deviation of 29 7 μg/100 ml The mean level on the 10th day was also significantly different from the basal level ($p < 0.01$) On the 14th day, two thirds of the subjects showed a higher level than basal one case showed no change and two cases showed a slight decrease in level The overall average change from basal level was + 11 1 with a standard deviation of 17 8 μg/100 ml However the mean value for the 14th day level was not significantly different from the basal level mean at the 5% level ($p > 0.05$)

DISCUSSION

In the present work both groups of infants and children vaccinated against small pox and measles showed significant rise in plasma glucocorticoids

After small pox vaccination, there were significantly higher levels of plasma 17 OHCS on the fifth and tenth days with no difference between the mean levels of plasma 17 OHCS on these days (Table 1) However, some cases showed higher levels on the fifth day while others on the tenth day This might be related to the severity of the reaction to the vaccination at the time the blood samples were taken Usually small pox vaccination gives rise to general and local reactions after the development of viremia around the fifth day (2) The

Table 2 *Plasma 17 OHCS levels before and after measles vaccination (μg/100 ml)*

Subject	Basal level	Level after vaccination 7th day	10th day	14th day
1	20	40	18	21
2	26	52	22	20
3	12	63	48	15
4	13	13	13	—
5	20	60	48	20
6	5	70	68	46
7	9	90	—	25
8	24	62	109	64
9	16	39	72	14
10	10	66	46	—
11	5	35	26	12
Mean	14 5	54 4	47 0	26 3
S D	7 2	20 8	29 8	17 3

reaction reaches its maximum around the tenth day This was the reason for selecting the fifth and tenth days for the plasma glucocorticoid determinations

In children vaccinated against measles estimation of plasma 17 OHCS levels was performed on the seventh tenth and fourteenth days after vaccination The seventh to the tenth day period after measles vaccination represents the incubation period which is shorter than in natural measles (4 5 15) The clinical manifestations which may be observed in response to measles virus vaccine as fever coryza and exanthema appear on the seventh to the tenth day after vaccination and remain for two to three days The third estimation of plasma glucocorticoid was done for the follow up In the present series of children vaccinated against measles (11 cases) four cases developed mild fever coryza and mild exanthema for two to three days about one week after vaccination The remainder of the cases showed no clinical reaction

In the whole group of children vaccinated against measles except in one case there was a rise in the plasma 17 OHCS level on the seventh tenth and fourteenth days after vaccination (Table 2) The rise on the seventh and tenth days was significant while that on the fourteenth day was not significant The mean level was higher on the seventh day than that on the tenth day however the difference was not significant There was no correlation between the plasma 17 OHCS levels obtained and the presence or absence of clinical reactions to measles vaccine

There is no laboratory data available in the literature on the response of the adrenal cortex to vaccination procedures However the results of some recent studies carried out on children vaccinated against measles might be considered as an indirect evidence of hyperactivity of the adrenal cortex after this procedure (3 5 6 10 15)

In the light of the results obtained in the present study small pox and measles vaccinations could be considered as stressful procedures acting upon the hypothalamic pituitary adrenal axis leading to its hyperactivity Accordingly it would appear that an increased corticosteroid administration is to be recommended following measles and small pox vaccination to all children having adrenocortical insufficiency

SUMMARY

The adrenocortical glucocorticoid function was studied in two groups of children 1) Children vaccinated against small pox and 2) Those vaccinated against measles Results obtained showed that there was a significant rise in plasma glucocorticoid levels following these vaccinations

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THE CAT EYE SYNDROME
REVIEW AND TWO FURTHER CASES OCCURRING IN FEMALE SIBLINGS WITH NORMAL CHROMOSOMES

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Considerable difficulty is sometimes experienced in categorizing an individual manifesting a complex combination of congenital anomalies particularly when many of the defects are common to a number of syndromes each of which incorporates an accepted spectrum of variability Clinical classification is frequently facilitated however by comparing the case in question to those already documented Cytogenetic studies also assist resolution in those instances associated with a chromosomal abnormality Conversely an apparent failure to define a chromosomal aberration when it is expected is to say the least confusing and necessitates a comprehensive reappraisal Such was the problem with the presented cases

These female siblings share many structural defects and the patt rn of malformation is consistent with that of the cat eye syndrome The cases of this syndrome reported to date (4 6 8 11 15 19 23 32 34) with only one exception (15) have had a small extra acrocentric or submetacentric chromosome present

Therefore the determination of normal female karyotypes initially detracted from the clinical diagnosis and another diagnosis was sought by reviewing those syndromes in which al atresia is described All this exercise appeared to achieve was a confirmation of the original proposition

CASE REPORTS

Case 1

The elder the firstborn child of healthy parents aged 27 and 28 was d livered following a surgical induction in November 1968 at 41 weeks gestation There is no known parental consanguinity The pregnancy during which ferrous gluconate a multivitamin preparation and standardised senna were taken was without incident apart from mild toxaemia in the last weeks of the third trimester

The body dimensions were a birth weight of 2 810 g (tenth percentile) (3) a vertex to heel length of 48.5 cm (third percentile) and a fronto-occipital head circumference of 32 cm (less than third percentile) Numerous abnormalities were evident the ears were small and low set with shell shaped pinnae and bilateral pre auricular skin tags The eyes were small with epicanthic folds and horizontal palpebral fissures The bridge of the nose was flat and broad and the palate was high arched The anus was atretic with the rectum opening into the vestibule just anterior to the fourchette Both feet were in calcaneo-valgus and the hands whi h had single palmar creases, were deviated radially with th wrists held in flexion On the second day a cardiac murmur was heard without cyanosis or evidence of congestive heart failure The murmur persisted and a clinical diagnosis of the Tetralogy of Fallot was made later in infancy

The abnormal positioning of the hands and f et slowly resolved though difficulties in abducting the hips prompted radiological examination This showed an increased acetabular angle of the left hip joint and a delay in maturation of the left upper femoral epi



Fig 1 Facies of case II Note the corneal opacity and underlying coloboma

physis suggesting instability of the joint Subsequent examinations of the eyes confirmed the microphthalmia and hypertelorism demonstrated circular pupils and the absence of corneal and lenticular opacities Unfortunately the ocular fundi were never adequately visualised to allow comment on the posterior poles however fixation and following were not positively evident at 3 months of age

The infant's growth and psychomotor progress were poor she was severely retarded physically mentally deficient and microcephalic Later in infancy a scoliosis of the thoracic spine developed and her general state of health progressively deteriorated She died of broncho pneumonia when aged 13 months

At necropsy the external features were as previously described the Tetralogy of Fallot was confirmed and an atrial septal defect demonstrated The kidneys and urinary tract were normal

Case II

The second child was born in January 1971 two weeks beyond the expected date of delivery The pregnancy labour and delivery were uneventful The only drugs taken during pregnancy were ferrous fumarate folic acid and calcium carbonate

This infant had a birth weight of 2610 grams (tenth percentile) a vertex to heel length of 47 cm (less than third percentile) a fronto-occipital head circumference of 31 cm (less than third percentile) and displays many structural defects Both ears are low set and abnormally shaped and there are bilateral pre auricular skin tags Hypertelorism a left epicanthic fold and bilateral microphthalmia are evident The left eye has a corneal opacity in the upper lateral quadrant beneath which is a coloboma of the iris The palpebral fissures have a mongoloid slant and the palate is high arched (Fig 1) The anus is atretic and a recto-perineal fistula is present (Fig 2) Both feet are in calcaneo-valgus and dislocation of the left hip joint was demonstrated radiologically The hands have single palmar creases and the index and little fingers have fixed flexion deformities of the distal interphalangeal joints The nails of these fingers and also of the little toes are hypoplastic An isolated systolic murmur was heard at the initial examination this has persisted and the diagnosis of Tetralogy of Fallot subsequently made

Regular review of this child has confirmed the microcephaly and demonstrated that both her physical growth and psychomotor development are grossly retarded

INVESTIGATIONS

Laboratory tests conducted on both infants included blood count urine analysis and bacterial inhibition tests for some serum amino acids (phenylalanine methionine leucine tyrosine histidine) these were all within the normal range Skull chest pelvic and limb radiology in both cases revealed only the hip pathology and a cardiac contour consistent with the cardiological diagnosis An intravenous pyelogram on



Fig 2 The perineum of case II

Table 1 *Incidence of anomalies reported in two or more cases of the cat eye syndrome*
+ = present − = absent ? = suggestive m = mosaic others not stated in reports

Feature	References														Present cases	
	1			2	3	4		5	6	7	8	9	10	11	I	II
Sex	F	F[b]	F	F	M	M	M	I	F	F	F	F	M	M	F	F
Ano-rectal anomaly	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Antimongoloid slant		+		+	+			+	+					+	−	−
Hypertelorism		+	+	+					+				+	+	+	+
Microphthalmia	+				+			+							+	+
Coloboma	+	+	+		−	+		+	+	+	+		−	+	−	+
Low set ears				+	+			+		+			−		+	+
Abnormally shaped ears				+	+								+	+	+	+
Preauricular tag/fistula	+	+		+	+	+		+	+	−	+	+		+	+	+
Oro-palatal anomaly				+	+					+				+	+	+
Cardiac defect				+	−	+	+			+				−	+	+
Renal anomaly			+	+	+	+							+	−	−	−
Psychomotor retardation		+	+	+		+	+	−					+	?	+	+
Physical retardation		+	+	+				−					−		+	+
Skeletal anomaly			+	+				+	+	+		+	+	+	+	+
Single palmar creases					+					+					+	+
Extra chromosome	+	+	+	+	+	m	+	+	+	−	+	+	+	+	−	−

(1) Schachenmann et al (2) Zellweger et al (3) Ishmael & Laurence (4) Gerald et al (5) Weber et al (6) Pfeiffer et al (7) Neu et al (8) Darby & Hughes (9) Curcio (10) Beyer et al (11) *Noel & Qua k*

[b] This case has also been reported by Schmid (24) who recorded the psychomotor and physical retardation

the second infant demonstrated normal renal anatomy and function

CYTOGENETIC STUDIES[1]

Cyto ogical preparations were made *from peripheral* blood leucocyte cultures using a modification of the method of Moorh ad et al (14) Skin fibroblast cul tures were carri d out on both ca es u ing *the method* of Harnden (10) Autoradiographic studies of b ood leucocyte cultures from the second ca e *were done* using a continuous labelling technique described by Gilbert et al (9) and quin crine fluorescent staining was done using the method of Pearson et al (18)

Thirty metaphases were examined from both the blood and skin cultures of Ca e I Th karyotype was 46 XX and no abnormalities were present Leucocyte and fibroblast cultures from Case II showed no abnormality in the 30 metapha e cells studied from each culture and metaphases from a s cond leucocyte *cul ture* labelled with tritiated thymidine also showed a normal karyotype The fluorescent banding *pat erns* of th chromo omes in 8 karyotypes showed perfect pairing betw en homo ogues thus ruling out the pres ence of a major hidden translocation *Thirty meta* phases from leu ocyte cultures of each parent were studied and showed no abnormalities

DERMATOGLYPHICS

This investigation had not been conducted on Case I In Case II the patterns of the fing r and palm prints are striking in their simplicity Simple arches some of which are almost flat are seen on 7 digits Both ring fing rs have an ulnar loop and the left midd e finger has a whorl Both axial triradii are displaced distally and towards the ulnar border of the palm In the right palm th triradial height is 38 % of the palm length and in the left it i 36% The b and d triradii are missing in the left palm and in the right the d tri radius is absent Both hypothenar patterns are carpal arche There are open fields in both thenar areas

The d rmatoglyphi s of a previous case (6) showed the predominance of whorls in th finger prints and in another (5) all ulnar loops were seen The axial *triradii* were distally placed in the e two patt nts as in the present cas but this is a rather non specific finding

Not enou h is known about the dermatoglyphic patterns in the cat-eye syndrome to draw any useful conclusions about the diagnostic contribution of th patterns in the present case

[1] Human Genetics Research Unit

DISCUSSION

The similarity between these siblings is strik ing and they would therefore appear to be long to the same clinical syndrome The ma jority of the defects evident are "non specific in that they are common to a number of documented syndromes However the one dif ferentiating feature appears to be the anal



Fig 1 Facies of case II Note the corneal opacity and underlying coloboma

physis suggesting instability of the joint Subsequent examinations of the eyes confirmed the microphthalmia and hypertelorism demonstrated circular pupils and the absence of corneal and lenticular opacities Unfortunately the ocular fundi were never adequately visualised to allow comment on the posterior poles however fixation and following were not positively evident at 3 months of age

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Fig 2 The perineum of case II

therefore reasonable in this instance to either accept the proposal of Neu et al consider the presence of a hidden translocation not detectable by quinacrine staining or suggest that the phenotype of the cat eye syndrome may be associated with a normal chromosome constitution from conception

SUMMARY

Female siblings both displaying anal atresia eye and ear defects retardation of growth and psycho motor development skeletal abnormalities and a congenital cardiac lesion are presented This anomaly complex is very suggestive of the cat eye syndrome Subsequent failure to demonstrate the extra chromosome associated with this entity appears to discount the diagnosis However after considering the numerous alternatives and offering explanations for the normal cytogenetic findings this diagnosis is proposed by the authors

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atresia with rectal fistula formation Anorectal abnormalities are not uncommon, the incidence being approximately 1 per 1 000 to 5 000 Caucasian live births This malformation is frequently associated with anomalies of other organs, particularly the genito urinary tract the heart, and atresia or stenosis of the gastro intestinal tract Numerous authors (12 25 26 27 30, 33) have demonstrated a familial incidence though in these instances the lesion has been isolated The defect is a major component of the cat eye syndrome (4, 6, 8 11 15, 19 23 32, 34) and the polydactyly/imperforate-anus/vertebral anomalies syndrome (p /i a /v a syndrome) (13, 21, 22 29) It has also been reported occasionally as sociated with trisomy E (31) and the 13q-deletion (Dq) syndrome (1) the ring D chromosome (Dr) syndrome (1) and the syndrome of caudal regression (7)

With reference to the described cases the presence of multiple anomalies is at difference with previous reports of familial anal stenosis and rather suggests a syndrome complex The syndrome of caudal regression is rejected on clinical grounds and all the remaining syndromes presented for consideration with the exception of the p /i a /v a syndrome, have an associated chromosomal abnormality, although Szotowa & Kowalewska (28) described a chromosomally normal male infant clinically resembling the trisomy E syndrome and a case of the cat eye syndrome with normal chromosomes has been reported (15)

Many of the listed anomalies have been observed in patients with trisomy E and Dq and Dr syndromes however the described pattern of malformation is not characteristic of these entities and failure to demonstrate the relevant chromosomal derangement most probably precludes these possibilities The alternative diagnosis therefore entertained as most tenable, is either the cat eye syndrome with a normal karyotype or a variant of the p /i a / v a syndrome

Review of the published data on the p / i a /v a syndrome (13 21 22, 29) discloses an almost constant combination of a pre axial defect (polydactyly or ectrodactyly) vertebral and rib anomalies and imperforate anus associated with other sporadically occurring anomalies involving the heart renal tissue alimentary system and facies That the presented cases fail to meet the defined criteria is evident, and this diagnosis is therefore discounted

Twelve cases of the cat eye syndrome (4 6 8, 11 15 19 23 32 34) together with 2 other possible cases (2 17) have been collected from the literature These are summarised in Table 1 which records recurring anomalies and also includes the considered siblings

As can be seen the anomalies frequently found are anal atresia eye and ear defects cardiac lesions mental retardation and skeletal anomalies although many others have been reported

Evaluation of the presented cases on a comparative basis supports the clinical diagnosis though failure to define the associated chromosomal aberration is disconcerting

Gerald et al (8) described a case exhibiting the complete syndrome who is a mosaic for the extra chromosome and Neu et al (15) reported the cat eye syndrome in a 3 ½ month old girl with a normal karyotype This report however has since been challenged though not on cytogenetic grounds (20)

The demonstration of normal chromosomal complements in the siblings and parents may mean that the cytogenetic studies conducted simply failed to disclose the abnormal cell line or as Neu et al (15) suggest this abnormal cell line bearing the extra chromosome may have disappeared after having exerted its teratogenic effect during organogenesis This explanation Neu et al (16) had previously presented when describing the disappearance of the trisomic leucocyte cell line in a 46 XX/ 47 XX C+ mosaic

That a significant mosaic state has been inadvertently overlooked is doubted and it is

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ANTIBODIES IN HUMAN MILK AGAINST E COLI OF THE SEROGROUPS MOST COMMONLY FOUND IN NEONATAL INFECTIONS

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Since breast milk antibodies are not resorbed by the infant (18) their protective role has been brought into doubt The dominating immunoglobulin of milk as well as of other secretions secretory IgA is according to recent concepts (17 6) a mediator of local immunity however Therefore a protective role of milk antibodies in humans should be sought on the mucous membranes of the gastrointestinal tract In accordance with such a local function of milk antibodies is the observation that they seem to protect against gastrointestinal infections (10 16) Furthermore a recent study has shown that breast milk consumption could be of significance for the protection against neonatal septicaemia (19) where the infection route often is reported as unknown (2 14 15 19) but may be through the intestinal wall

To further analyse the possible importance of breast milk antibodies in relation to neonatal infections we have in this preliminary report studied the occurrence in breast milk and in stools of neonates of antibodies against the O antigens of the *E coli* serogroups most commonly encountered among strains causing neonatal infections (2)

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MATERIAL AND METHODS

Serum samples were obtained at parturition from healthy mothers and infants delivered after full term normal pregnancies Consecutive samples of stool from the infants and of breast milk were taken during the following days If not processed immediately they were kept at −20 C

Antibody determinations were mad by indirect haemagglutination using as antigen a pool of O antigens representing eight of the most commonly found *E coli* serogroups (1) The results were expressed as reciprocal titres The stool specimens from the infants were handled as described by Kenny et al (9) The milk samples were centrifuged (3 000 rpm for 30 min) prior to titration Reduction with mercaptoethanol of milk and serum specimens was perform d as earlier described (7)

RESULTS

In milk samples from 17 out of 19 tested mothers antibody titres of 2 000 or more were found with the O antigen pool used From the initial high levels there was a downward trend in most cases (Fig 1)

Consecutive stool samples from seven infants were also tested with significant amounts of antibodies found in five of them Their highest reciprocal titres were 128 128 2 000 4 000 and 4 000 All had consumed high titrered breast milk in amounts adequate for their age as in the example shown in Fig 2 *a* One of the two infants without coproantibodies

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Key words *Ano-rectal anomaly familial incidence* cat-eye syndrome phenotype with apparently normal karyotype

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ANTIBODIES IN HUMAN MILK AGAINST *E COLI* OF THE SEROGROUPS MOST COMMONLY FOUND IN NEONATAL INFECTIONS

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Since breast milk antibodies are not resorbed by the infant (18) their protective role has been brought into doubt The dominating immunoglobulin of milk as well as of other secretions secretory IgA is according to recent concepts (17 6) a mediator of local immunity however Therefore a protective role of milk antibodies in humans should be sought on the mucous membranes of the gastrointestinal tract In accordance with such a local function of milk antibodies is the observation that they seem to protect against gastrointestinal infections (10 16) Furthermore a recent study has shown that breast milk consumption could be of significance for the protection against neonatal septicaemia (19) where the infection route often is reported as unknown (2 14 15 19) but may be through the intestinal wall

To further analyse the possible importance of breast milk antibodies in relation to neonatal infections we have in this preliminary report studied the occurrence in breast milk and in stools of neonates of antibodies against the O antigens of the *E coli* serogroups most commonly encountered among strains causing neonatal infections (2)

MATERIAL AND METHODS

Serum samples were obtained at parturition from healthy mothers and infants delivered after full term normal pregnancies Consecutive samples of stool from th infants and of breast milk were taken during the following days If not processed immediately they were kept at −20 C

Antibody determinations were mad by indirect haemagglutination using as antigen a pool of O antigens representing eight of the most commonly found *E coli* serogroups (1) The results were expressed as reciprocal titres The stool specimens from the infants were handled as described by Kenny et al (9) The milk samples were centrifuged (3 000 rpm for 30 min) prior to titration Reduction with mercaptoethanol of milk and serum specimens was performed as earlier described (7)

RESULTS

In milk samples from 17 out of 19 tested mothers antibody titres of 2 000 or more were found with the O antigen pool used From the initial high levels there was a downward trend in most cases (Fig 1)

Consecutive stool samples from seven infants were also tested with significant amounts of antibodies found in five of them Their highest reciprocal titres were 128 128 2 000 4 000 and 4 000 All had consumed high titrered breast milk in amounts adequate for their age as in the example shown in Fig 2 *a* One of the two infants without coproantibodies

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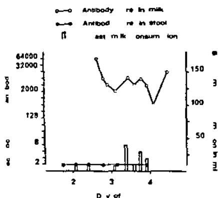


Fig 2b A 68 05 13 Similar diagram as in Fig 2*a* from another mother-infant pair where no *E coli* antibody titres appeared in the infant s stool in spite of high titres in the maternal milk presumably due to the low breast milk consumption of the neonate

body synthesis by the child cannot be ex cluded however

The demonstration of the resistence of the coproantibodies to proteolytic degradation and low pH (9) fits well with the observation that the dominating immunoglobulin of milk (4) and other secretions (6 17) secretory IgA is a compact molecule quite resistant to the pro teolytic activities of gastrointestinal juice (3) The study of Kenny et al (9) as well as this study does not show whether or not the titres found in milk are wholly or partly due to se cretory IgA antibodies In unpublished experi ments we have seen equally high coli antibody titres in secretory IgA isolated from human colostrum however (8)

The fact that the milk antibodies to *E coli* do not appear in the infant s serum as in many other species (5 18) does not speak against their protective role Since secretory IgA seems to mediate local immune defense of mucous membranes (6 7) milk *E coli* antibodies may protect the gastrointestinal mucosa lo cally This would be a practical way of helping the neonate to meet with the challenge of the *E coli* strains colonizing the gastrointestinal tract after birth

With regard to the suggested increase in the frequency of *E coli* colonization (12) and in fection (11) in maternity wards and newborns the possible protective role of breast milk anti bodies may become of greater importance More detailed studies are necessary to analyse the presumed function of breast milk anti bodies in neonatal *E coli* colonization and in fections

SUMMARY

In this preliminary study antibodies against the O antigen of some of the most common serotypes of *E coli* were demonstrated in breast milk usually in high titres Those in fants who had consumed significant amounts of breast milk with high titres of *E coli* anti bodies had such antibodies in their stool The possibility that these antibodies which pre-sumably are of the secretory IgA type may protect the gastrointestinal mucosa of the neo nate against microbial invasion should be fur-ther investigated

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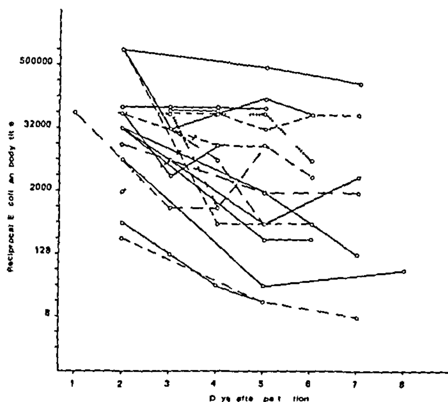


Fig 1 Th course of *E co i* anti body titres in consecutive samples of breast milk A pool of O anti gens representing the eight most prevalent O groups of *E coli* was used for the titrations

had consumed only small amounts of milk (Fig 2 *b*) whereas in the other case the milk was low titrered (1/256–1/16)

Serum antibodies against the *E coli* O anti gen pool were determined in cord blood from 22 infants Two had a titre of 1/16 and the others were 1/8 or below These antibodies were resistant to reduction with mercapto ethanol

DISCUSSION

It is obvious from the present study that high titres of antibodies are found in human milk against O antigens representing the serogroups of the *E coli* strains which caused 31 out of 43 instances of *E coli* infections in neonates (2) These antibodies appeared in the stool in sig nificant amounts as earlier reported for anti bodies against enteropathogenic *E coli* (9) and against *Salmonella* (13) The fact that in infants with low intake of high titrered breast milk or high intake of low titrered milk no or low antibody titres were detected indirectly suggests that the antibodies were derived from the milk This is further supported by the low titres seen in the infant s sera A local anti

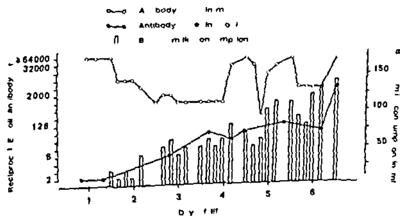


Fig 2 a G 68 06 03 The antibody titres in a mother s milk and the infant s stool against the pool of eight O antigens The appearance of high titres in the stool with increasing breast mil

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DISTRIBUTION OF DIVALENT CATIONS AT THE CELLULAR LEVEL DURING PRIMARY HYPOMAGNESEMIA IN INFANCY

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During the last few years attention has been drawn to a new disease in infancy characterized by primary hypomagnesemia with secondary hypocalcemia and apparently due to an isolated malabsorption of magnesium from the gastrointestinal tract (1 10 16 21 22 23 28 29 32 33 35) This disease offers a unique possibility to study the role of magnesium on electrolyte homeostasis at the cellular level In adults a magnesium deficiency is secondary to a predisposing disease or condition and the development of a severe magnesium deficiency following dietary magnesium depletion is slow (30) In infants however a much greater demand for magnesium is due to rapid growth Therefore the effect of a negative magnesium balance such as in primary hypomagnesemia is more pronounced and magnesium deficiency develops rapidly

In the present study the changes of cations in the extracellular and cellular compartments have been studied in an infant with primary hypomagnesemia The results show important differences in the distribution ratios of magnesium and calcium at the cellular membrane level during magnesium substitution and during withdrawal periods The mechanisms behind such differences are discussed on the basis of enzymatic processes regulating cellular electrolyte homeostasis The role of magnesium on parathyroid hormone action is also considered

PATIENT AND METHODS

Our patient is a girl (C. L. 681231) who was admitted at th age of 2 months because of generalized convulsions None of the more common reasons for convulsive disease at this age was found and conventional therapy had no effect Initially she had a borderline hypocalcemia but in spite of calcium administration her serum calcium fell further to subnormal levels and her convulsions persisted She developed a generalized oedema with rapidly expanding head circumference due to fluid accumulation intracranially At this stage a profound hypomagnesemia (0 5 mEq/l) was found and i m injections of magnesium sulfate momentarily abolished her seizures A normalisation of her serum calcium followed whereas the serum magnesium remained low (1 0–1 5 mEq/l) Long term therapy with magnesium gluconate by mouth at high dosage (30 mEq/day) was started and has up to now been continued for 2 / years with an entirely normal somatic and psychomotoric development

During several periods a withdrawal of magnesium substitution was tried but was followed by a reappearance of hypomagnesemia and hypocalcemia within a few days When the girl was 22 months of age the magnesium substitution was withdrawn for a period of 3 weeks Prior to this and at the end of th withdrawal period skeletal muscle biopsies were taken from the lateral part of the quadriceps femoris muscle according to the technique of B rgstrom (2) Similar samples were taken from normal children of corresponding age The samples were weighed immediately (Cahn RG automatic electrobalance) and weight losses during the weighing procedure were corrected for by extrapolation to wet weight at zero time The biopsy samples were then dried in an oven to constant weight After fat extraction with petroleum ether the fat free dry weight could be recorded Such samples were then extracted with a known volume (50 μl) of 2 N HNO for 5 hours after which the potassium and sodium content could be analysed by micro flame photometry (13) The magnesium cal

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Key words Breast milk antibodies colostrum antibodies coproantibodies *E coli* antibodies

Of special interest is the relation between the distribution changes of the two divalent cations magnesium and calcium In the controls the Mg /Ca ratio is 0 38 The corresponding ratio for the patient is 0 30 during magnesium substitution during the withdrawal period this ratio falls by 60% to 0 12 which is less than 1/3 of the controls The availability of magnesium at the external cell membrane is very low during magnesium depletion In the controls approximately three times more calcium than magnesium is available if one considers the total concentrations In the patient with primary hypomagnesemia the concentration of calcium at the cell membrane is approximately 8–9 times that of magnesium In the intracellular compartment no such dramatic change takes place in the relations between the two divalent cations During substitution the Mg_i /Ca_i ratio is 6 7 and after 3 weeks of magnesium withdrawal the corresponding value is still 6 5

DISCUSSION

The clinical course in this girl is in agreement with that of previously reported cases of primary hypomagnesemia in infancy It is noteworthy that neither the convulsions and general neurological hyperirritability nor the hypomagnesemia did respond to intravenous calcium infusions On the other hand magnesium administration promptly abolished the clinical manifestations and also brought about a rapid normalization of the plasma calcium level Balance studies have suggested a specific defect of the intestinal absorption of magnesium as the prime cause of the disease whereas the calcium balance remains positive (10 20 32 33) There have been no indications of an exaggerated magnesium loss On the contrary the renal conservation is augmented with only a negligible urinary excretion of magnesium The hypothesis of an isolated magnesium malabsorption therefore appears justified

The wellknown intimate relationship between magnesium and calcium homeostasis becomes particularly interesting in states of magnesium depletion The present investigation shows that within a short time period there is a drastic fall of magnesium in the extracellular fluid The intracellular content of magnesium and also the distribution of calcium across the cell membrane is much more stable These changes imply a relative excess of calcium ions at the outer cell membrane In controls the calcium/magnesium ratio was about 3 while in the magnesium depleted child this ratio increases to about 8 to 9 Since the fraction of ultrafiltrable magnesium and calcium remains mainly unchanged during hypomagnesemia (36) the availability of calcium ions in relation to magnesium ions at the outer cell membrane increases about three times Supposing an approximately equal affinity of membrane sites for magnesium and calcium (5) then the possibility for magnesium binding decreases considerably Membrane ATPase systems for active transport of potassium and sodium across the cell membrane are inhibited by calcium but stimulated by magnesium (11 12 15 31) Under such circumstances the active transport of potassium and sodium will be less active during hypomagnesemia (8) with a loss of intracellular potassium and a gain of intracellular sodium (see Table 2) The intracellular content of magnesium and calcium remains much more stable and there is no indication of any significant change in the calcium/magnesium ratio Both these ions appear to exist in a bound form to a great extent i e retained by cellular structures and/or present inside a permeability barrier e g in the mitochondria (24 27) At the internal cell membrane the relative availability of magnesium and calcium remains largely unchanged unless there is a shift from free to bound fractions Therefore the changed availability of these ions at the outer cell membrane must play the major role for the changes seen in cellular potassium and sodium homeostasis

The second main aspect of magnesium de

Table 1 *Changes of electrolyte concentrations in plasma following withdrawal of magnesium supplement in primary hypomagnesemia*

	Before	After	Change (%)
Mg (mEq/l)	1 3	0 5	−62
Ca (mEq/l)	5 2	4 0	−23
K (mEq/l)	4 5	3 9	−13
P (mg/100 ml)	4 8	6 0	+25

cium and chloride content was determined by micro flame emission spectrophotometry (14) The chloride content was used as an estimate of the extracellular phase of the muscle samples The intracellular elec trolyte content was calculated assuming that there was a Gibbs Donnan distribution between plasma and ex travascular fluid

Plasma electrolyte changes and the renal excretion of calcium magnesium and phosphorus were followed during the withdrawal period The calcium potassium and sodium content of these fluids were determined by flame photometry (Eppendorf) and the magnesium content by atomic absorption spectrophotometry (Uni cam SP 90) Phosphorus in plasma and urine was de termined by the method of Fiske Subbarow

RESULTS

The effects of a three week magnesium withdrawal on serum electrolytes are shown in Table 1 Magnesium fell to values around 0 5 mEq/l already after 1 week and remained at this low level for the rest of the withdrawal period The change of serum calcium was even more rapid, and within 3 days calcium had fallen from 5 2 to about 4 2 mEq/l However the relative change of calcium was less prominent than that of magnesium, being 23% and 62% respectively Serum phosphorus rose continuously during the period of observation At the same time the urinary excretion of magnesium decreased to negligible amounts (about 0 1 mEq/24 hrs) and the same was true for calcium (about 0 1–0 2 mEq/24 hrs) Only a small reduction of the urinary clearance of phosphorus was observed (20–30%) The serum protein content was unchanged (about 7 5 g/100 ml) and no blood pH changes were observed during the withdrawal

Table 2 *Mg Ca K and Na in mEq/l of intra cellular water of skeletal muscle*

Samples taken before and at the end of a three week period of withdrawal of magnesium supplement are compared with similar samples from eight controls

I c /e c ratios are based on simultaneous determinations in intracellular water and plasma

	Patient			Controls (n = 8)	
	Before	After	Change (%)	Mean value	S D
$Mg_{i c}$	20 0	18 1	− 9 5	21 9	±0 9
i c /e c	13 3	36 2	+172 0	11 5	
$Ca_{i c}$	2 98	2 79	− 6 4	3 71	±0 4
i c /e c	0 60	0 66	+ 10 0	0 74	
$K_{i c}$	108 9	101 3	− 7 0	114 0	±3 3
i c /e c	27 2	26 0	− 4 4	28 5	
$Na_{i c}$	15 2	23 2	+ 53 0	7 6	±0 4
i c /e c	0 107	0 163	+ 52 0	0 054	

The analytic variation for K and Na is 2–3 % (13) for Ca and Mg 3–4% (14)

The electrolyte concentration in skeletal muscle before and at the end of the magnesium withdrawal period is presented in Table 2 and compared with similar determinations on samples from control patients The concentration of magnesium calcium and potassium in muscle cells was slightly lower in the girl with primary hypomagnesemia already at the outset when a daily magnesium supplement of 30 mEq was still given In contrast to this the sodium concentration was considerably higher The withdrawal of magnesium was followed by a further decrease of the potassium magnesium and calcium concentrations and in a further increase of the cellular sodium concentration

Of chief interest however is the distribution of these cations on both sides of the cellular membrane When the intracellular values are compared with the simultaneous plasma concentrations important changes in the concentration gradients between the muscle cell and the plasma are revealed Thus the i c /e c ratio for magnesium is increased by about 170% Corresponding changes in calcium relations only amount to an increase of 10% The potassium i c /e c ratio shows a small reduction, whereas that of sodium increases by about 50%

Of special interest is the relation between the distribution changes of the two divalent cations magnesium and calcium In the controls the Mg_e/Ca_e ratio is 0 38 The corresponding ratio for the patient is 0 30 during magnesium substitution during the withdrawal period this ratio falls by 60 to 0 12 which is less than 1/3 of the controls The availability of magnesium at the external cell membrane is very low during magnesium depletion In the controls approximately three times more calcium than magnesium is available if one considers the total concentrations In the patient with primary hypomagnesemia the concentration of calcium at the cell membrane is approximately 8–9 times that of magnesium In the intracellular compartment no such dramatic change takes place in the relations between the two divalent cations During substitution the Mg_i/Ca_i ratio is 6 7 and after 3 weeks of magnesium withdrawal the corresponding value is still 6 5

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RESULTS

The effects of a three-week magnesium withdrawal on serum electrolytes are shown in Table 1 Magnesium fell to values around 0 5

Table 2 *Mg Ca K and Na in mEq/l of intracellular water of skeletal muscle*

Samples taken before and at the end of a three week period of withdrawal of magnesium supplement are compared with similar samples from eight controls

I c /e c ratios are based on simultaneous determinations in intracellular water and plasma

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ic/ec	13 3	36 2	+172 0	11 5	
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ic/ec	0 60	0 66	+ 10 0	0 74	
K_{ic}	108 9	101 3	− 7 0	114 0	±3 3
ic/ec	27 2	26 0	− 4 4	28 5	
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the outer cell membrane Mg depletion and a relative excess of calcium ions leads to decreased stimulation of the membrane bound adenyl cyclase system

ACKNOWLEDGEMENTS

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AGE DEPENDENT REACTIONS OF RECTAL AND SKIN TEMPERATURES OF INFANTS DURING EXPOSURE TO COLD

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The heat emission of the skin of newborn infants was earlier recorded thermographically (14) The comparatively high temperature of the nape and interscapular region noticed during cold exposure has been referred to cold stimulated metabolism of subcutaneously situated brown adipose tissue (8 14 15) The special heat production of this organ so called non shivering thermogenesis probably forms an important heat source in human infants during the neonatal period (4 11 16) In certain newborn mammals there is a gradual involution of brown adipose tissue parallel to a diminishing dominance of non shivering thermogenesis (2 6 18) An age associated decrease in intensity of the heat radiation of the nape skin of human infants might indicate a declining activity of underlying brown adipose tissue

In human infants the capacity of peripheral vasoconstriction is well developed from birth on (3 17) but the cold induced rise in oxygen uptake is not as pronounced in the first postnatal hour as later (4) In case the oxygen supply of the body is not adequate to the demand such an extra metabolic activity as non shivering thermogenesis might be limited

The aim of this study was to find out if the low capability to balance heat loss in the first postnatal hour is accompanied by a low heat emission of the nape region and if simultaneously with an improving resistance to cooling there is an increased heat release from this region Moreover it was of interest to know if the intensity of the cold associated heat emission of the nape would decline later after birth

Thus the changes in rectal temperature and skin temperatures of the back with special regard to the nape were registered in infants during 30 min of exposure to low ambient temperature at different ages in the first postnatal hours and in the first 6 months of life

This study was supported by grants from Semper Fond for Näringsforskning and Karolinska Institutets Reservationsanslag

MATERIAL AND METHODS

The material consisted of 58 fullterm healthy infants all vaginally delivered and with an Apgar score of 8 or more at 1 and 9 or more at 4 min Data on the infants are given in Table 1 The parents were informed and had no objections to the study The 78 examinations performed were divided into 7 groups according to the age of the included infants

1 The change in skin and rectal temperature during 30 min of cooling in the first postnatal hours

Infants of *groups 1 and 4* were born into room temperature (approx 24 C) immediately dried and wrapped in a sheet After about 2 min they were put in a bed and covered with blankets Within 10 min the examination of babies of *group 1* started in the room of thermographic scanning and it continued until 30 min after birth The infants of *group 4* were brought to the nursery to be washed weighed meas

Table 1 *Data on infants studied*

Group	No	Birth weight (g)	Weight when studied (g)	Treatment before cold exposure	Exposed to cold at an age of (mean)	Thermo-graphy	Skin temp by el thermo-meter
1	10	2 490–4 200	The ame	0	0–30 min	10	10
2	10	2 990–3 690	The same	Protected from cooling[a] for 40 min from birth on	40–70 min	10	10
3	8	2 980–4 370	The same	Protected from cooling[a] for 75 min from birth on	75–105 min	8	5
4	8	2 760–4 540	The same	Routine handling after birth and in nursery Put in bed until 2 hrs of age Kept in incubator[a] for 30 min	145–175 min	8	8
5A	10	3 240–4 020	3 050–3 900	From postnatal wards half an hour after a meal Protected from cooling[b] for 30 min	1–4 days (3)	10	10
5B	10	1 970–4 370[c]	2 110–4 030	From postnatal wards or homes Undressed	4–14 days (7)	10	5
6	10	1970–4 370[c]	3 100–5 200	From their homes Undressed	1–2 mo (51 d)	10	10
7	12	2 840–4 120	6 000–8 900	From their homes Undressed	3–6 mo (4 7 m)	12	6
					Sum	78	64

[a] Kept undressed in an incubator (Isolette Air Shields) of an air temperature of 33–35 C
[b] Kept undressed in an incubator (Isolette Air Shields) of an air temperature of 32–33 C
[c] One infant with intra uterine growth retardation Vitality high

ured dressed and put in bed with water bottles (approx 38 C) and blankets At a m an age of 2 hours they were placed naked in the incubator of 33 to 35 C and kept there for 30 min before the exposure to low ambient temperature began

The infants of *groups 2 and 3* were first protected from cooling Thus at birth they were quickly placed on bottles with hot water (approx 38 C) with an infrared lamp heating from above They were immediately dried and wrapped in warm sheets and within 2 min placed in the incubator with an air temperature of 33 to 35 C There they were kept for 40 and 75 min respectively after which time the cold exposure began

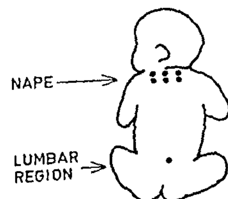


Fig 1 The points on the back of the baby measured with skin applicator of the electrical thermometer

Infants of *group 5 A* were kept for 30 min in the incubator with an air temperature of 32 to 33 C prior to th cold exposure Some reactions of these babies have been discussed earlier (14) Four of these infants were also participants of group 1

? The change in cold stimulated heat emission of the back skin during the first months of life

Group 5 B consisted of 10 infants studied at a mean age of 7 days Five of these babies had also been looked upon 75 min after birth (group 3) All of them were again studied at an age of 1 to 2 months (*group 6*) *and one of them finally participated in group 7* which included 12 babies at an age of 3 to 6 months

The infants of groups 5 B 6 and 7 were exposed to cold immediately after undressing and the study began about one hour after a meal

The babies of groups 1 to 6 were exposed to an ambient temperature of 22 to 23 C a degree of cooling which should cause a maximal stimulation of the heat producing system in the early postnatal period (4) During examination of the oldest babies (group 7) many of them being twice as heavy as at birth the ambient temperature was kept at about 18 C

Technique of temperature registration

During the 30 min of cold exposure the infants were kept naked on their abdomen on a bed in front of the thermographic camera unit A mirror reflected the infrared emission of the body surface of the baby into the camera unit situated at a distance of about

Table 2 *The mean rectal temperature before and after 30 min of exposure to an ambient temperature of 22 to 23 C and the mean rate of fall per min during this period in infants studied at various times after birth*

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1	0–15 min	37 6	36 1±0 1	0 10	$p<0\ 001$
	15–30 min	36 1±0 1	35 4±0 1	0 05	
2	40–70 min	37 1±0 2	36 0±0 1	0 04	$p<0\ 001$
3	75–105 min	37 0±0 1	36 2±0 1	0 03	$p<0\ 001$
4	145–175 min	36 5±0 1	36 1±0 2	0 01	$p<0\ 02$
5A	1–4 days	36 6±0 1	36 4±0 1	0 00	
5B	4–14 days	36 7±0 1	36 6±0 1	0 00	

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Skin temperature (skin applicator H) and rectal temperature at a depth of about 6 cm were m as ured with an electrical thermometer type Ellab H (Copenhagen) The points recorded are een in Fig 1 In accordance with the terminology used in an earlier thermographic study (14) the area between the neck and scapulae is referred to as th nape The mean of 6 measurings in the nape was used for cal culations

It was earlier noticed that in infants being kept in heat gaining environment prior to the cold exposure skin temperature of the whole back surface dropped in the first period of cooling During the last 15 to 20 min however temperature of the nape skin remained constant or increased at the same time as lower back areas continued to cool down (14) Thus in order to re ister the heat gaining pha.e of the nape skin tem peratures were measured after 15 and after 30 min of cold exposure Rectal temperature was measured be fore coolin, and after 15 and 30 min of cold exposure

RESULTS

In the infants cooled from birth on rectal tem perature fell within 15 min from an intra uterine level of at least 37 6 C (19) down to 36 1 C (Table 2) The later after birth the ex posure to cold began the slower was the rate of fall in rectal temperature during cooling Thus whereas the average drop was 0 1°/ min in the first 15 min after birth it was only 0 03 /min during cooling in the second post natal hour (Table 2) The difference in tem perature decline during cooling the first 30 postnatal min and during cooling the second

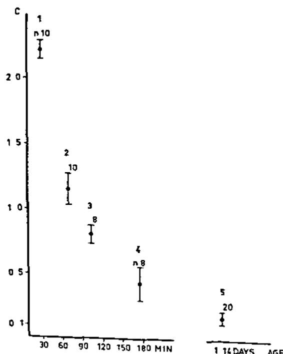


Fig ? The change (=drop) in rectal temperature of infants during exposure to an ambi nt temperature of 23 C for a period of 30 min at various times after birth Mean±S E

Table 1 *Data on infants studied*

Group	No	Birth weight (g)	Weight when studied (g)	Treatment before cold exposure	Exposed to cold at an age of (mean)	Thermo-graphy	Skin temp by el thermo-meter
1	10	2 490–4 200	The same	0	0–30 min	10	10
2	10	2 990–3 690	The same	Protected from cooling[a] for 40 min from birth on	40–70 min	10	10
3	8	2 980–4 370	The same	Protected from cooling[a] for 75 min from birth on	75–105 min	8	5
4	8	2 760–4 540	The same	Routine handling after birth and in nursery Put in bed until 2 hrs of age Kept in incubator[a] for 30 min	145–175 min	8	8
5A	10	3 240–4 020	3 050–3 900	From postnatal wards half an hour after a meal Protected from cooling[b] for 30 min	1–4 days (3)	10	10
5B	10	1 970–4 370[c]	2 110–4 030	From postnatal wards or homes Undressed	4–14 days (7)	10	5
6	10	1970–4 370[c]	3 100–5 200	From their homes Undressed	1–2 mo (51 d)	10	10
7	12	2 840–4 120	6 000–8 900	From their homes Undressed	3–6 mo (4 7 m)	12	6
					Sum	78	64

[a] Kept undressed in an incubator (Isolette Air Shields) of an air temperature of 33–35 C
[b] Kept undressed in an incubator (Isolette Air Shields) of an air temperature of 32–33 C
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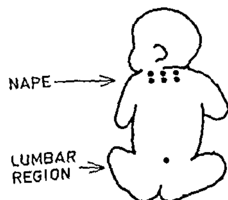


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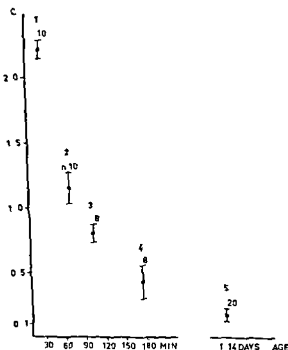


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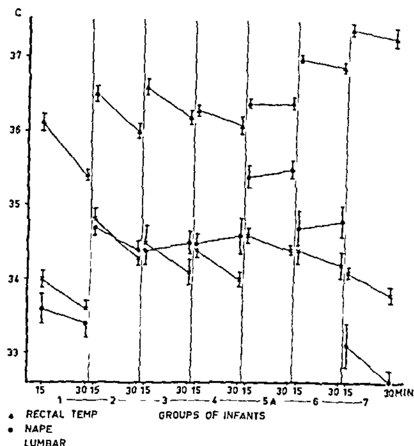


Fig 3 The change in rectal nape and lumbar skin temperatures during the last 15 min of exposure to low ambient temperature in infants of various ages Mean SE

half hour after birth was highly significant ($p < 0\ 001$)

The increased resistance to cooling was obvious in those infants studied twice in the first postnatal days Four infants were examined from birth on and again within 4 days and 5 infants were examined in the second hour after birth and again within 6 days The mean change in rectal temperature of these babies at each occasion did well correspond to that of the total group studied (Fig 2 cf 1 and 5 3 and 5) The experimental conditions differed however, in that the babies had been fed the second time At an age of 1 to 14 days no significant change in rectal temperature occurred during 30 min of exposure to 23°C ambient temperature

Fig 3 shows the mean temperature changes of rectum and of the nape and lumbar skin during the last 15 min of exposure to cold An age associated increase in the level of rectal temperature is apparent after the first day of life (5 A, 6, 7) The mean nape temperature is lower than the lumbar one and both of them decrease during cold exposure in the youngest and also in the oldest babies After 2 hours of extra-uterine life and still at an age of 1 to 2 months (mean 51 days) the nape region is warmer than the lumbar one and a slight temperature gain occurs there during cooling (groups 4 5 A 6)

The age associated variation in behaviour of the nape region is further elucidated in Fig 4 The increasing positive gradient between the nape and lumbar skin temperature in infants of groups 2 to 5 is mainly caused by the rise in temperature level of the nape skin The differences between infants younger than 3 hours infants 1 to 5 days and infants 3 to 6 months old are highly significant

The number of babies possessing a warm area in the nape region and the number of recorded infants with a temperature gain of this patch during the last 15 min of cooling are summarized in Table 3 A difference of 0 5°C or more between adjacent areas caused a distinct contrast on the thermal picture Temperature gain was defined as a rise of at

least 0 2 C of a mean value of 2 closely situated measured points within the warm patch seen on the thermogram As earlier emphasized the mean change in nape temperature of each group of infants was not significant (14) However the frequent tendency to heat gain in the nape within a certain age period contrasting to the cooling off of lower back regions is very impressive (Table 3) In the majority of infants a decrease in temperature of the lumbar area was seen during cooling

Typical patterns of thermographically recorded cold associated heat emission of the back skin of babies of various age are demonstrated in Fig 5 After cold exposure for 30 min in the first postnatal hour the skin temperature was very low and evidently there was a delay or lack of vasodilatation of the nape region (A B) In the second hour of life the skin remained warmer during cooling but curiously enough there was a prominent cool zone in the nape (C) which later generally had got the highest heat emission after cooling (F showing the same baby as C) From the third postnatal hour on the heat release of the nape

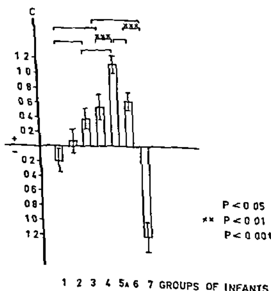


Fig 4 The nape lumbar skin temperature gradient after 30 min of exposure to low ambient temperature of infants of various ages Mean ± S E.

Table 3 *The frequency of a warm patch in the nape after 30 min of cold exposure and the number of those infants measured (64) in whom a heat gain of this area was observed during the last 15 min of cooling*

	A Thermographic recording		*B* Skin temperature control by electrical thermometer	
Time of cooling	No of infants	Warm patch in the nape ( )	No of infants	Temperature gain of the warm patch ( )
During the first post natal hour	20	25	20	15
During the sec post natal hour	8	40	5	20
During the third post natal hour	8	90	8	60
At an age of 1 14 days	20	100	15	90
At an age of 1–2 months	10	80	10	70
At an age of 3–6 months	12	0	6	0

Warm patch An area, distinctly outlined on the thermogram as its temperature was at least 0 5 C higher than that of surrounding surfaces
Heat gain A rise of at least 0 2 C of the mean value of 2 measured points within the warm patch

and interscapular region dominated (D E F G) At an age of 4 to 6 months no warm patch in the nape was observed (H and I)

In all babies studied a second or third time at a mean age of 51 days a warm area in the nape was still present but had decreased in size and temperature level (Fig 5 cf F and G Fig 6 A and B) Four months after birth the nape was cooler than lower back surfaces (Fig 6 C)

In 14 infants there appeared a quiver in the thigh and thorax muscles during cold exposure This activity which might reflect the shivering thermogenesis was seen in 10 of the babies younger than 2 days Four of these were cooled during the first 30 min of life

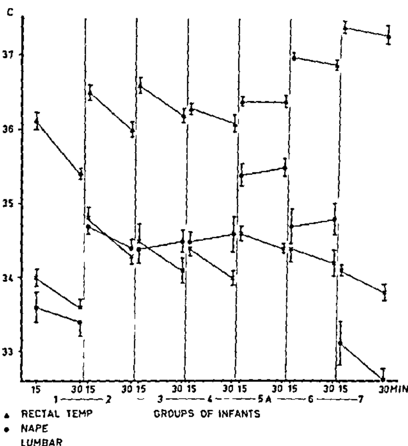


Fig 3 The change in rectal nape and lumbar skin temperatures during the last 15 min of exposure to low ambient temperature in infants of various ages Mean S E

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cooled after a period of at least 30 min in warm environment The rate of temperature fall seems to be more dependent on age when cooling than on the level of rectal temperature at the start of cold exposure (Table 2)

Thermal instability of newborn infants has been repeatedly attributed to great heat loss due to poor insulation and a large body surface (1 4 5) As these conditions do not change in favourable direction during the short period discussed here other factors than a diminishing heat loss must contribute to improve the defense against cooling Though the age associated increasing basal metabolic rate lowers the critical temperature below which an extra metabolism is needed to keep the body temperature constant (10) it hardly explains the improving resistance to a more intense cooling This is probably due to a growing capability of the baby to increase the oxygen uptake to meet high demands A certain rise in oxygen uptake due to cold stimulation has been found to occur at least 15 min after birth (4) According to Pribylova et al 2 ½-hour-old babies are able to increase the oxygen uptake sufficiently to maintain rectal temperature constant during one hour of exposure to an ambient temperature of 24 C (13)

During delivery mechanical compression gives rise to an oedema of the skull which impairs heat penetration from deeper tissues and thus is outlined thermographically by low heat emission (17) The cold nape of infants recorded earlier (17) and again in this study during the first postnatal minutes could hardly be explained in a similar way as this area is not exposed to such a pronounced pressure as the caput succedaneum

The low temperature of the nape skin and the lack of cold stimulated heat gain seen there in the majority of babies younger than one hour might well be a consequence of the maximal vasoconstriction which occurs in order to diminish radiation heat loss (3) With increasing heat production the constriction of skin vessels should be counteracted

It cannot be excluded that the initial low

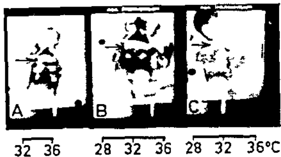
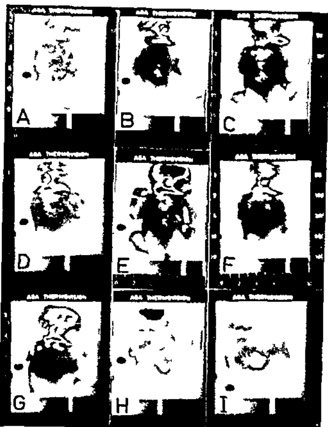


Fig 6 The pattern of heat emission of one infant after 30 min of exposure to low ambient temperature at 3 different occasions In all thermograms the warmest skin areas of the back are covered by saturated white As the scale covering 10 C was differently adjusted from time to time the temperatures are indicated below the thermograms A At *2 weeks* of age the warmest area is formed by the nape and a rather symmetrical wing like configuration B At *one month* of age the warm area in the nape is still prominent though it is cooler than at the first occasion The patches in the scapular region of the same temperature as the nape are now smaller compared to those seen at an age of 2 weeks C At an age of *4 months* the nape is not the warmest area any more Note the low temperature

heat emission of the nape as well as the simultaneously occurring drop in rectal temperature is due to a reduced ability to increase oxygen uptake It is known that the cold stimulated rise in metabolic rate in newborn mammals is particularly sensitive to hypoxia (9) Thus at this first postnatal period an insufficient oxygen supply of the body might selectively cause a low oxygen saturation of thermogenic organs such as brown adipose tissue and thus inhibit the heat releasing oxidative metabolism there

The special area sharply defined from surrounding surfaces by its more intense heat emission that was seen in the nape during cold exposure of most infants from the third hour on was still present about 2 months after birth This observation supports results of Silverman et al who recorded a comparatively high temperature of the nape of babies as old as 78 days (15) The repeated examinations of the same infants during the first months of life performed in the present study revealed however that the warm area diminished in size



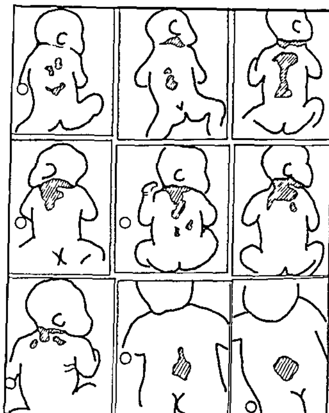


Fig 5 Thermographic presentation of the back skin of infants of various ages after exposure to low ambient temperature for 30 min

The thermograms are presented invertedly and thus warm areas are shown dark and cold areas are shown white With the aid of the built in isotherm function patches of saturated white are superimposed on the thermal picture On the scale adjusted to cover 10 C (between 27 and 37 C) the right hand edge of the white marker indicates the temperature of the areas covered by the isotherm The black dot to the left is the black body reference temperature set to 37 C

In all photo the *warmest* regions of the backs of the infants have been marked In all the drawings the warmest parts of the backs of the babies are marked with striped patches to help make clear the areas of highest temperature The heat emission of the skull being lower in babies with hair than in bald ones has been left out of consideration

A and B Neither after the cooling in the *first half hour* (A) nor after cooling in the *second half hour* (B) after birth the nape region is the warmest one on the back C After cold exposure in the *second postnatal hour* the neck and scapular regions have got about the same temperature The area in between that is the nape is cooler however D In the *third postnatal hour* the neck nape and interscapular regions are warmer than the surrounding E The baby of (A) is here *4 days old* In the nape and interscapular region there is a patch of about 35 C which is warmer than surrounding back areas F The infant of (C) is now *6 days* old The nape being cooler than the surrounding at the earlier occasion is now the warmest place on the back G The infant of (C) and (F) now *6 weeks* old The distribution of heat is close to that found at 6 days of age (F) but the temperature level is somewhat lower and the size of the warmest patch seems smaller H and I At an age of *4* and *6 months* respectively the area on the back with the high st skin temperature is found in the lumbar region

DISCUSSION

With thermography a rapid drop in skin temperature beginning in the periphery has been observed in human infants immediately after birth (17) Heat loss due to evaporation of amniotic fluid probably contributes to the quick cooling off of the skin, thus reducing the skin environmental temperature gradient (1)

The rates of fall of rectal and skin temperatures during the first 15 min after birth do well correspond with those observed by Gandy et al (7) A declining fall of rectal temperature with time after birth was evident in Gandys neonates being continuously exposed to room temperature from birth on for more than one hour The same tendency was noticed in our series of

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Key words Newborn infants rectal temperature skin temperature thermography brown adipose tissue non shivering thermogenesis

and intensity and finally disappeared The declining intensity of heat radiation of the nape might be explained by reduction of thermal conductance, caused by growing layer of subcutaneous white adipose tissue The decrease in size of the warm patch is more likely due to a change in vaso resistance or vascularity of cutaneous or subcutaneous tissues in this area It has been shown that there is a decrease in amount of typical' brown adipose tissue of children during the first year of life (12) With the anatomical distribution of brown adipose tissue in mind, one can hardly exclude the possibility of the observed age associated phenomena reflecting the metabolic activity of brown fat and some gradual change in this organ

SUMMARY

At 78 occasions 58 healthy fullterm infants of an age of 0 to 6 months were exposed to an ambient temperature of 18 to 23°C for 30 min Thermographic recordings were made and dorsal skin and rectal temperatures measured

During the first hours after birth there was a negative relationship between age when cooling and rate of fall of rectal temperature Simultaneously there was a positive relationship between age when cooling and level of skin temperature of the nape region

In the first postnatal hour a cold associated decrease in heat emission of the nape was generally observed However during cooling in the subsequent 2 hours a slight heat gain of the nape became more frequent

In most infants from the third postnatal hour on and at least until the age of 2 months the warmest area on the back was found in the nape, where also a slight temperature rise often was seen during cooling However, there was an age associated decrease both in the intensity of heat emission and size of this area At 4 months of age the nape was cooler than lower parts of the back

The possibility of the heat emission of the nape reflecting thermogenesis of underlying brown adipose tissue and age associated changes in activity and/or amount of this tissue is discussed

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14 Rylander E Pribylová [illegible]

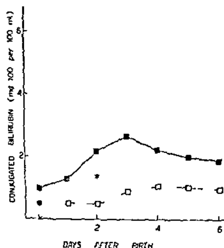


Fig 1 Conjugated bilirubin in serum from newborns of mothers treated with immunosuppressive drugs and from newborns of untreated mothers during the first days of life ■ Conjugated bilirubin in n wborns suffering from erythroblastosis foetalis □ conjugated bilirubin in newborns suffering from erythroblastosis foetalis who were born to mothers treated with immunosuppressive drugs $p<0.01$ ● $p<0.05$

zyme catalysing the conversion of tryptophan into formyl kynurenine is present in immature babies at birth as well as in full term infants and increases to a very high level within a few days of delivery (1) On the other hand it is generally accepted that the enzymes may be activated by an increase in their own substrate This is probably also valid for bilirubin UDP glucuronyl transferase

There are some drugs for example phenobarbital which are able to increase the activity of many different hepatic enzymes The mechanism of this action is to be explained on the basis of an induced *ex novo* synthesis of enzymatic molecules in the microsomes

On the other hand it has been conclusively demonstrated that novobiocin is able to inhibit bilirubin UDP glucuronyl transferase activity especially in newborns

Immunosuppressive drugs are known to provoke a condition of hyperbilirubinemia in some cases of hepatic insufficiency They act by inhibiting protein synthesis and in certain cases even cause hepatic necrosis (12) In all the present cases even if the immunosuppressive drugs lowered the Coombs titre in some cases total bilirubin was not significantly different from that of the control group (11) In spite of these findings the level of conjugated bilirubin is lower in newborns of mothers treated with immunosuppressive drugs These results could be explained on the basis of the inhibition in the foetus of *ex novo* enzymatic protein synthesis (bilirubin UDP glucuronyl transferase enzyme) by immunosuppressive drugs substances which are able to pass across the placental barrier (13) These findings which were obtained by clinical studies seem to demonstrate that the foetal activation of bilirubin UDP glucuronyl transferase by unconjugated bilirubin even in the heaviest immunized infants may be partially inhibited by treating the mothers with immunosuppressive drugs for various medical indications

SUMMARY

Studies of conjugated bilirubin levels in newborns suffering from erythroblastosis foetalis

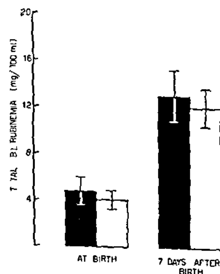


Fig 2 Total bilirubin at birth and at 1 week of age in infants born to moth rs treated with immunosuppressive drugs and in infants born to untreated mothers ■ Total bilirubin in newborns suffering from erythroblastosis foetali □ total bilirubin in newborns suffering from erythroblastosis foetalis who were born to mothers treated with immunosuppressive drugs

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SERUM CONJUGATED BILIRUBIN IN NEWBORNS OF MOTHERS TREATED WITH IMMUNOSUPPRESSIVE DRUGS

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Various authors have reported findings which indicate that only unconjugated bilirubin is present in funicular blood (3 4) They attempt to explain this fact with the claim that the hepatic enzyme bilirubin UDP glucuronyl transferase is inactive at birth (7)

Recently, Bakken (3) and other authors (2 5, 6) have demonstrated that foetal bilirubin UDP glucuronyl transferase is activated by the increased unconjugated bilirubin found in foetuses suffering from erythroblastosis foetalis The author has also found similar results based on studies of the funicular plasma level of conjugated bilirubin found in newborns affected by this disease (9)

In this investigation the level of total and conjugated bilirubin is reported in infants suffering from erythroblastosis foetalis who were born to mothers treated with immunosuppressive drugs by the obstetrician in order to lower the plasma level of the circulating antibodies (13, see also 10 11) The drugs, ametopterin and 6 mercaptopurine were administered to the mothers only after the twentieth week of gestation

MATERIALS AND METHODS

The subjects studied in this report consisted of a group of 10 full term infants suffering from erythroblastosis foetalis due to Rh immunization The anti D titres were determined in the mothers during pregnancy by means of the indirect Coombs test The maximum Coombs titre was between 256–512 in all cases Total bilirubin and conjugated bilirubin were determined according to Malloy & Evelyn (8)

Six infants born to mothers treated with ametopterin (2 5 mg per day) and 4 infants born to mothers treated with 6 mercaptopurine (100 mg per day) were examined

The control group consisted of 10 full term newborns with erythroblastosis foetalis whose mothers were not treated with immunosuppressive drugs during pregnancy The Coombs titre level of this second group was in the same range as that of the experimental group

RESULTS

The amounts of conjugated bilirubin found in infants born to mothers treated with immunosuppressive drugs and in infants born to untreated mothers are shown in Fig 1 The difference between the two curves gives an indirect evaluation of the hepatic bilirubin UDP glucuronyl transferase activity In fact a significant difference was already demonstrable at birth The maximum difference between the two curves was reached between the third and sixth day after birth

Fig 2 shows the levels of total bilirubin found in the infants at birth and at 1 week of age of mothers treated with ametopterin or 6 mercaptopurine as well as those of untreated mothers These levels are not significantly different

DISCUSSION

Many hepatic enzymes which were previously thought to be inactive during foetal development have recently been shown to be active at birth even in immature infants

For example it has been demonstrated that tryptophan oxygenase activity, the liver en-

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SOME EFFECTS OF RUBELLA VACCINATION ON IMMUNOLOGIC RESPONSIVENESS[1]

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Some viral diseases such as measles chicken pox and rubella are known to depress cutaneous delayed hypersensitivity to tuberculin (2 5 14) and the same effect has been observed after administration of live viral vaccines (3 7 8) Recently it was also demonstrated that during measles viral hepatitis and after vaccination against measles there occurs a depression of blast transformation of lymphocytes cultivated in vitro in the presence of phytohaemagglutinin (PHA) Such lymphocytes cannot be activated by tuberculin (6 7 9) It would appear that these effects depend on a temporary impairment of lymphocyte functions possibly triggered by the virus (11) Lymphocytes from infants with congenital rubella were demonstrated to lack the property of being activated by PHA (11 12)

During a trial with rubella vaccine (4) we have studied its effect on the tuberculin reaction on the peripheral leukocytes and on the blast transformation of lymphocytes of the vaccinees

MATERIAL AND METHODS

Eight tuberculin positive children seronegative to rubella antigen in good general condition and not treated with immuno-suppressive drugs were investigated Each child received a dose of 1 ml of live viral vaccine Cendehill (lot 8 K 25 Rb 11) intramuscularly HAI antibodies were titrated both before vaccination and after 7 14 24 and 40 days *ad modum* Stewart et al (13) Each child received 5 units of purified tuberculin intradermally in a volume of 0 1 ml before vaccination and 7 14 and 24 days thereafter to test delayed hypersensitivity Total and differential leukocyte counts were carried out in each child before the vaccination and after 7 14 and 24 days Lymphocyte cultures were mad in each subject before the vaccination and 7 14 and 24 days after th vaccination by the mi romethod of Hungerford as modified by Kravis et al (10)

This work was supported by the Italian National Research Council (CNR)

Kindly supplied by R I T., Genval Belgium

RESULTS

Four of the 8 patients demonstrated a transitory depression of the tuberculin reaction 7–14 days after the vaccination Two subjects (R S and R R) showed a change in the type of cutaneous reaction (Table 1) Before the vaccination the tuberculin reaction was strongly positive with a blister in these two patients In the first case the reaction was the same after 7 days but not after 14 and 24 days In the second case blisters did not appear in the three tests carried out after the vaccination After 24 days the diameter of the reaction was larger than before the vaccination in 3 patients In all cases 7–14 days after the vaccination a decrease of the total leukocyte counts ($p < 0\ 001$ at 7 days and $p < 0\ 01$ at 14 days) was observed along with a decrease of peripheral lymphocytes and eosinophils (Table 2) The blastic transformation of lymphocytes in the presence of old tuberculin was

born to mothers treated with ametopterin and 6 mercaptopurine seem to demonstrate that the foetal activation of bilirubin UDP-glucuronyl transferase by unconjugated bilirubin may be partially inhibited by treating the mothers with immunosuppressive drugs In fact the level of conjugated bilirubin is lower in newborns of mothers treated with these drugs in comparison with those of the control mothers

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Key words Bilirubin newborn immunosuppressive drugs erythroblastosis foetalis enzyme activation

ment in our vaccinated patients was supplied by the study of blast transformation of lymphocytes chiefly in the presence of tuberculin antigens In our cases the difference in response between the skin test and the blast formation can be explained by the fact that the former is a complex reaction based on various types of cells and humoral factors whereas the latter is an in vitro phenomenon based on a limited and selected cellular population

The depression of cutaneous delayed hypersensitivity and decrease in number of peripheral leukocytes can be explained by a temporary migration of white cells from the periphery stimulated by the viral antigen

The possibility of an increased endogenous corticosteroid production has also been considered as an explanation of the decrease in the tuberculin reaction in the course of some viral disease Corticosteroid production however was found to be normal in children during measles (1) and also the live measles vaccine had no effect on the immediate cutaneous hypersensitivity reaction to ragweed and house dust allergens in three patients studied by Fireman et al (7)

We must also consider the possibility suggested by some authors that the decreased responsiveness to tuberculin could be dependent upon the repeated intradermal injections with this antigen This hypothesis could be ruled out since our cases showed 24 days after the vaccination a tuberculin skin test with an induration similar and even larger than that obtained prior to the administration of the vaccine

SUMMARY

Eight tuberculin positive children sero negative against rubella were vaccinated with a live attenuated rubella vaccine

A temporary depression of the peripheral lymphocyte functions was observed

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Key words Rubella vaccination blastic transformation of lymphocytes tuberculin skin test

Table 1 *Average diameter (in mm) of induration of tuberculin reaction before and after the rubella vaccination*

Name	Sex	Age	Diameter before vaccination (mm)	After vaccination (days) 7	14	24
R S	F	8	13 5[a]	13 5[a]	12	18
P M	F	5	14 5	14	16	15
R R	F	3	14[a]	14	16 2	14
D G F	F	2	18 5	10 5	7	13
D G G	F	5	18 5	6 5	18	16 5
M R	F	6	11 5	11 5	10	15
M M	M	7	12 5	11	13	11 5
C M	M	11	13	12	13	18

[a] With blister

Table 2 *Mean value of leukocyte counts in the peripheral blood of the 8 children studied before and after the rubella vaccination*

	Cells × 10³/mm³ (mean ± S D)			
Leukocytes	Before vaccination	After vaccination (days) 7	14	24
Total	7 9±1 2	6 2±1	6 6±1 1	7 4±1
Lymphocytes	4 7±0 4	3 2±0 7	3 3±0 7	4 1±0 3
Neutrophils	2 7±0 3	2 7±0 4	3 1±0 5	2 8±0 4
Eosinophils	0 5±0 08	0 3±0 05	0 2±0 05	0 5±0 07
t for coupled data on total leukocyte counts		5 97735 $p<0.001$	5 17402 $p<0.01$	1 82662 n s

depressed in all of our 8 patients 7 days after the vaccination ($p<0.01$) (Table 3) We observed the same phenomenon in the presence of purified tuberculin ($p<0.02$)

In the presence of PHA the depression of blast formation was weaker (n s) (Table 3) Spontaneous blast formation in the control tubes was never higher than 2% Six of our eight vaccinated children became seropositive against rubella antigen 24–40 days after the vaccination

DISCUSSION

The depression of the tuberculin skin test and the decreased number of leukocytes, lymphocytes and eosinophils are probably correlated phenomena the former depending on the latter Lymphocytes are well known as mediators of the delayed type of hypersensitivity The tuberculin reaction was depressed in only half of our cases and not in all as expected A similar result in rubella vaccinated children was observed by Lamb (8)

A better index of the lymphocyte involve

Table 3 *Blastic transformation of lymphocytes in presence of old tuberculin purified tuberculin and PHA in the 8 children vaccinated with rubella vaccine*

	Blasts ( ) Old tuberculin Before vaccination	Days after vaccination 7	14	24	Purified tuberculin Before vaccination	Days after vaccination 7	14	24	PHA Before vaccination	Days after vaccination 7	14	24
R S	24	14	28	26	47	17	36	26	44	47	30	44
P M	18	5	17	13	25	16	30	18	26	20	18	36
R R	20	9	18	18	40	18	30	28	30	28	28	36
D G F	31	4	2	17	39	15	5	40	22	18	18	33
D G G	16	13	22	20	31	19	33	33	43	42	35	39
M R	23	10	24	24	21	18	25	19	24	16	20	32
M M	19	12	14	22	36	36	18	31	30	30	29	24
C M	16	12	10	19	15	10	12	11	24	19	25	26
Mean S D	21 ±5	10 ±3	17 ±8	20 ±4	32 ±10	18 ±6	23 ±9	27 ±6	30 ±8	28 ±9	26 ±6	33 ±5
t for coupled data		4 147 $p<0.01$	1 037 n s	0 467 n s		3 387 $p<0.02$	1 735 n s	2 256 n s		2 236 n s	2 398 n s	−1 493 n s

Table 1 *The frequency of signs of CNS involvement in 237 cases of incontinentia pigmenti collected from the literature*

Dominant sign	No of cases	of 237 cases
Cerebral palsy	28	1
Convulsions	21	9
Mental retardation	24	10
Other symptoms	19	8
Total	88	39

greater slow activity from the right hemisph re had appeared on the EEG but no epileptogenic activity was seen

At 17 months of age convulsions have still not reappeared Her psychomotor development is nearly normal for her age and she still has no signs of spasticity

DISCUSSION

Incontinentia pigmenti was described for the first time probably at a meeting of the Clinical Society of London in 1905 by A E G Garrod (7) Adamson (1) presented another case in 1907 a mentally retarded girl with a generalised but not universal retiniform pigmentation In 1925 the disease was described both by Bardach (3) under the name of systematisierte Naevusbildungen and by Bloch (4) Bloch s patient was then more fully described by Sulzberger in 1927 (19) and the disease was given the name incontinentia pigmenti or the Bloch-Sulzberger syndrome

The first Swedish case reported was by Almqvist (cited by Sulzberger (19)) and later on other cases have been described from this country (13 15 16) A total of about 250 cases have been reported in the literature A comprehensive survey of the literature up to 1955 was produced by Wodniansky (20) and in 1959 Pfeiffer (18) made a comparison of 141 earlier cases and calculated the frequency of different symptoms of the syndrome Carney & Carney (5) have presented a large material of their own 26 cases Kitamura et al (11) account for 21 cases amongst the Japanese population Gordon & Gordon (8) have discussed different etiological factors in i p Details of the clinical picture of the syndrome are given in these papers

Symptoms indicating CNS involvement are common in i p A summary of these is given in Table 1 the patients have been collected from the authors cited above (5 11 18 20) Convulsions were thus the dominant symptom in 21 out of 237 cases When the convulsions have been described in more detail the most common types have been amorphous new born convulsions and later grand mal Only two of the cases described earlier have been associated with infantile spasms Bambridge (2) described the typical skin changes of i p in a girl in whom minor motor seizures occurring some 20 times a day appeared at 3 months of age No EEG study was carried out She was also mentally retarded had microcephaly and spastic tetraplegia Kellner & Kulz (9) reported an infant with repeated convulsions which appeared in the first week of life The EEG at 7 days of age showed a rapid basal activity with a spike focus in the left frontal region Three months later typische Blitz Nick und Salaam krampfe appeared An EEG now showed hypsarrhythmia ACTH treatment for several weeks gave no positive result the infantile spasms were followed by grand mal fits The patient presented in this paper had a normal EEG at 2 months of age Infantile spasms with a typical hypsarrhythmia pattern on the EEG appeared at 6½ months of age ACTH treatment according to a scheme recommended by Gamstorp (6) proved successful the convulsions disappeared as did the hypsarrhythmia in the EEG At 17 months of age convulsions had not reappeared The EEG at 13 months of age showed a slight side asymmetry but no focal or paroxysmal changes

The anatomical changes lying behind the symptoms from CNS in i p are essentially unknown O Doherty & Norman (14) describe a child who died at 7 weeks of age due to haemorrhagic gastroenteritis A prenatal malformation with micropolygyria of the cerebral cortex was found

I p is regarded by most authors to be geneti

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CASE REPORT

INCONTINENTIA PIGMENTI BLOCH SULZBERGERS SYNDROME, ASSOCIATED WITH INFANTILE SPASMS

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Incontinentia pigmenti (i p) is a rare disease, characterized by skin changes often combined with changes in the accessory skin organs Other organs are also involved teeth eyes and central nervous system (CNS) The CNS symptoms consist of different types of cerebral palsy mental retardation and convulsions About 250 cases of i p have been described in the literature, but only two of these have been associated with infantile spasm (2 9)

CASE REPORT

Girl born July 15 1970 No skin disease dental or neurological disturbances known among the relatives The mother had earlier given birth to a healthy girl but had also had three spontaneous abortions The pregnancy and delivery were normal the birth weight was 3 650 g length 49 cm

Already at birth changes in the skin were observed multiple areas of erythema thick walled vesicles on the lower part of the abdomen and on the extremities The vesicle content was yellow in colour No bacteria were found on culture of the vesicle content Attempts to isolate a virus from the vesicle content proved unsuccessful serological tests against herpes virus showed no increase in titre During the first 2 weeks of life new vesicles appeared especially on the abdomen and on the arms Some 10–14 days after their appearance these vesicles were transformed into the indurate stage with streaks on the skin arranged with no relation to the dermatomes or the innervation The peripheral *blood contained* up to 30 % eosinophilic leukocytes Histological examination of a skin biopsy with vesicle at 2 weeks of age confirmed th diagnosis of i p at the eruptive stage Another biopsy taken at 4 weeks of age agreed well with the indurate stage of i p An eye examination carried out at 4 weeks of age revealed superficial corneal changes bilaterally incipient bilateral cataracts and a pseudochoria optic on the right side An EEG at 2 months of age was normal

At $6\,{}^{3}/_{4}$ months of age she started to have typical attacks of infantile spasms with forward nodding of the head embracing movements of the arms and drawing up of her legs The child was whining and irritable The EEG now showed the typical pattern of hypsarrhythmia Treatment with ACTH 60 IU twice a day intramuscularly was commenced immediately after the EEG result was known 6 days after the convulsions began An unsuccessful treatment with pyridoxine was attempted prior to this The frequency of the fits decreased during the days following initiation of ACTH therapy and 5 days later the fits had ceased completely and have not as yet recurred After 2 weeks the ACTH dosage was reduced by 20 IU every third day A renewed EEG 11 days after the ACTH treatment began showed a significant improvement with no paroxysmal changes Another EEG after cessation of the ACTH treatment was nearly normal The patient was discharged from hospital at 7 / months of age

On examination at 9 months of age she had started to crawl but could not sit without support She had no signs of spasticity The skin was mostly in the verrucous stage of i p

On renewed examination at 13 *months* of age the patient s psycho-motor development was at the level of 10–11 months She had had no convulsions since the ACTH treatment The skin changes were in regression although some pigmented areas still remained Her teeth had started cutting at 9 months of age they were very sharp causing her to bite her tongue badly Her hair was sparse and straggly An eye examination revealed regression of the corneal opacities insignificant peripheral lens cloudiness remained Veil formed greyish membranes had appeared in the right vitreous humor A slight side asymmetry with a

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CASE REPORT

CONGENITAL CHLORIDE DIARRHOEA

Experiences with a New Case

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Congenital chloride diarrhoea (CCD) was first described in 1945 (1 6) Several reports have been published since then 25 cases are now known (11) The disease has previously not been reported from Norway While most of the reports have been on single cases the Finnish authors have been able to study a series of 13 cases and thus given valuable contributions to the understanding of this disease (9 10 11 13 15 16)

Clinical characteristics and pathogenesis

The mode of inheritance is autosomal recessive (11) Hydramnios may be present as a prenatal sign of CCD and in most cases the child is born before term Diarrhoea persists form shortly after birth Early signs of CCD may be hyperbilirubinemia and an excessive loss of weight during the first days of life The stools contain an excess of chloride the concentration stabilizing around 150 mEq/l after a few months The concentration of chloride in the urine is low The concentration of chloride in the stools and the urine seems independent of serum values and of chloride intake Hypoelectrolytemia develops rapidly primarily affecting the chloride concentration Hypokalemia and metabolic alkalosis develop later while sodium-concentration is better maintained Hyperaldosteronism is seen in several of the patients (7 9 13) Kidney biopsy has shown vascular and degenerative alterations (9 13) The disease is accompanied by a retardation of growth and motoric development CCD is also seen in adults (14)

A number of studies concerning the pathogenesis of CCD have been carried out in the patients previously described (1 2 3 4 6 9 13 14 17) These data suggest that the absorption of chloride is defective the defect substantially being found in the ileum and the colon It is believed that the primary defect is an error in the specific active mechanism of chloride absorption in the small and the large intestine (9 11) The Finnish authors have set forth the following hypothesis regarding the pathophysiological sequence in CCD the defective chloride absorption leads to depletion of electrolytes and water resulting in a hypovolemia The hypovolemia produces a secondary hyperaldosteronism which is responsible for the high urinary output of potassium and the hypokalemic alkalosis

CASE REPORT

A O was a boy born on March 26th 1969 He was the fourth child of a healthy mother His mother was remarried and he was the first child with her new husband His parents were unrelated The pregnancy was uneventful The boy was born 3 weeks before term a marked hydramnios was noted The birth weight was 3 000 g and the length 50 cm The lowest weight was 2 550 g (4 days old) Some abdominal distension and a moderate jaundice was noted during his first 5 days of life At an age of 2-3 weeks his mother noted that he had extraordinary watery stools and he

cally determined Palmgren (16) has however described a case where herpes simplex virus was isolated from the vesicle content An attempt to isolate this virus from the patient described here was not successful nor could an increase in titre against herpes simplex virus be obtained The majority of cases hitherto described have been girls, only 8 of 232 cases were boys (e g 10 15 17) It is also remarkable that there are relatively few brothers to the sisters affected Lenz (12) has put forward the hypothesis that i p is due to a mutation on the X-chromosome The affected females are heterozygotes Males with the mutated gene on their sole X-chromosome are more severely affected and generally die in utero Spontaneous abortions are in fact very common in families with a female member with i p The mother of the patient described here had had three spontaneous abortions before the affected girl was born

SUMMARY

A case is presented of incontinentia pigmenti Bloch–Sulzberger syndrome associated with infantile spasms Treatment with ACTH resulted in the disappearance of the convulsions and the EEG pattern of hypsarrhythmia also disappeared Ten months after the cessation of the convulsions she was still free from convulsions, had no signs of cerebral palsy or significant mental retardation

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Table 2 *Screening investigation of the family*

The baby brother was examined 4 days old The oldest brother was not available

	Father (5 9 15)	Mother (25 12 27)	Brother (6 10 53)	Brother (26 8 58)	Brother (25 1 71)
Blood					
pH	7 36	7 38	7 37	7 34	7 41
St bicarbonate	22	22	22	21	21
Base excess	−3	−2	−2	−3	−3
Serum					
mEq/l Na	140	144	140	144	128
mEq/l K	4 8	5 0	4 8	5 0	
mEq/l Cl	102	101	103	102	114
Urine					
mEq/l Na	120	105	80	80	16
mEq/l K	38	85	50	40	26
mEq/l Cl	105	89	88	86	22

and 2 mEq/l Small intestine biopsy immune globuline investigation and chromatography of amino acids were carried out without pathological findings Hormonal studies including determination of 17 keto and 17 ketogenic steroids and excretion of aldosterone in the urin were performed The two former were found within normal ranges the latter exceeding normal ranges for this age (6 3 μg/day)

Family Study

CCD is shown to be inheritable We were informed that the father of our patient presented a retarded motoric development and often had diarrhoea in childhood This encouraged us to carry out a screening investigation of the family Acid base balance serum electrolytes and urine-electrolytes were determined in all available members of the family (Table 2) No pathological values were found

DISCUSSION

The diagnosis of CCD seems evident on basis of the clinical and biochemical findings The treatment presented many problems During period I with massive supplement of electrolytes in i v infusion his condition deteriorated with increasing dehydration and diarrhoea contrasting his improved hypoelectrolytemia and metabolic alkalosis Transferred to a limited intake of electrolytes the opposite phenomenon was seen his clinical condition improved and the biochemical data worsened Several authors have previously noted that the diarrhoea seems dependent upon the excretion of chloride in the stools (3 4 7 12) Gamble observed that increased parenteral supplement of chloride might increase the diarrhoea (6) The condition of our patient was so bad that we feared a fatal outcome as the treatment was altered from period I to period II This unfavourable response to the treatment cannot easily be explained the main point being the rather paradoxical situation where the miserable condition of the patient was in striking contrast to the laboratory data It can however be concluded that a too vigorous correction of the electrolyte disturbances may be dangerous in these patients The depletion of electrolytes and the metabolic alkalosis must indeed be corrected but we feel that the acute crisis of our patient could have been avoided if the supplement of electrolytes primarily had been more moderate With the present supplement of chloride and potassium the patient manages well but he still has a metabolic alkalosis Theoretically this might be due to too small doses of electrolytes and it is to be hoped that the planned increase of K and Cl^- supplement will improve his alkalosis The aldosterone excretion being high he is suspected to have a secondary hyperaldosteronism

Table I *Laboratory investigations during the three different dietary periods*

Period I and II lasted 3 weeks each period III 5 weeks The electrolyte concentrations are determined in mEq/l Mean values during the periods are documented and the last values in the preceding period are especially noted to illustrate the development during each period

	Period I Cl 100 mEq/day K 100 mEq/day		Period II Cl 15–20 mEq/day K 25 mEq/day		Period III Cl 30–40 mEq/day K 25 mEq/day		
	Admission values 19 months	Mean values	Last values Per I	Mean values	Last values Per II	Mean values	Values 28 months old
Blood							
pH	7 55	7 45	7 44	7 60	7 65	7 50	7 54
St bicarb	42	28	25	45	52	30	32
Base Excess	+18	+ 7	+ 4	+20	+22	+ 8	+ 8
Serum-Na	138	140	140	134	134	140	140
Serum-K	2 5	3 8	4 9	3 0	2.8	3 5	5 0
Serum-Cl	72	95	98	70	66	90	94
Stools							
Cl-conc	148			140		135	
K-conc	20			20		15	
Na-conc	120			120		115	

continued to have such stools His motoric development seemed normal the first 6 months but gradually it became evident that it was delayed At the age of one year he could neither sit walk nor crawl As the months passed without any improvement of his skills he was admitted to the pediatric ward His diarrhoea had exacerbated during the last week before admission On admission he was 19 months old his weight was 8 300 g A marked muscular hypotonia was noted he could neither sit with support nor stand up He was seriously dehydrated with loss of skin turgor Table I shows the values of serum electrolytes and some acid-base parameters at the time of admission Parenteral fluid therapy was initiated in order to correct the metabolic alkalosis and the hypoelectrolytemia Fluid and electrolytes were administered in accordance with his clinical condition the degree of dehydration and the serum values of the electrolytes Initially (period I) he was supplied with 100 mEq of chloride and potassium per 24 hours On this treatment his metabolic alkalosis and hypoelectrolytemia improved (Table I) but his diarrhoea and dehydration increased Having doubled the volume and the number of stools per day he appeared critically ill After 3 weeks with this therapy the supplement of chloride and potassium was reduced (period II) Defecation became less frequent and the stools were more solid He obviously retained more water and his fluid balance was better These beneficial effects continued as we switched from intravenous to oral supplement of chloride (15 mEq day) and potassium (25 mEq/day of which 10 mEq as phosphate) However a gradual increase of the metabolic alkalosis and a gradual diminution of serum electrolytes was noted throughout the period With an increased supplement of chloride (30–40 mEq/day as KCl and NaCl) and continuous supplement of potassium (25 mEq day) his condition improved (period III) The laboratory data improved he gained weight and the stools were more solid and less frequent than in period I Receiving this treatment since then his motoric development has been satisfactory at 28 months he was able to sit and walk His general condition has been good the hypoelectrolytemia is improving but the metabolic alkalosis still remains

The concentration of chloride in the stools was in the different periods found fairly stable at 150 mEq/l but the chloride concentration did not constantly exceed the sum of Na^+ and K^+ (Fig 1) Chloride concentration in the urine was between 0

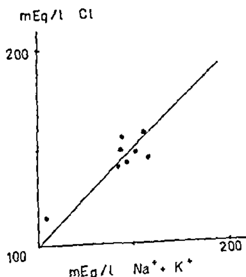


Fig 1 The relation between the output of chloride and the output of potassium and sodium in the stools

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CASE REPORT

INSULINOMA

Diagnostic Problems in a 12 year-old Boy

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Childhood hypoglycemia presents a very complex diagnostic problem It often remains unrecognized until alarming symptoms such as convulsions occur Furthermore it may be necessary to repeat tests periodically before the aetiology can be established

Functioning islet cell adenoma of the pancreas as a cause of childhood hypoglycemia is rare It has been reported in about 70 children (1) The diagnostic difficulties are demonstrated in the following case report

CASE REPORT

The patient was a boy aged 11½ years when he was admitted to another hospital in a coma During 9 months he had repeated episodes of restlessness irritability sweating and drowsiness A diagnosis of idiopathic hypoglycemia was made and treatment with frequent meals was started Four months later he was admitted to the Children s Hospital Basel for recurrent attacks of hypoglycemia At the time of examination the patient was 11 / years of age He showed normal physical and mental development without distinct clinical symptoms His height was 140 cm (= P) and he was slightly overweight (44 kg = P)

Diagnosis of hypoglycemia

Blood sugar varied between 8 and 30 mg/100 ml during clinical attacks of hypoglycemia. Determinations of the blood sugar over 24-hour periods showed wide fluctuations within one day as well as from day to day Values below 40 mg/100 ml were repeatedly found sometimes with minimal or no clinical symptoms (Fig 1)

Differential diagnosis of hypoglycemia

A decreased delivery of glucose has been excluded by a normal response of blood sugar to glucagon and

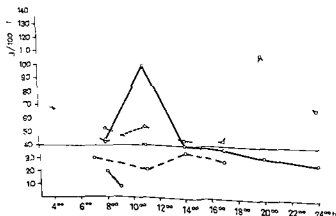


Fig 1 Serial blood sugar values over 24-hours before operation Different symbols represent various 24-hours periods

CONCLUSION

CCD must be considered in children with retardation of growth and motoric development, as has been described in this case During the course of hospitalization it was noted that his general condition deteriorated as we attempted to correct his electrolyte disturbances by massive i v infusions of chloride and potassium We are convinced that this could have been avoided if the electrolyte supplement had been more moderate Apart from this acute crisis the patient has confirmed previous experience that CCD can be treated satisfactorily with dietary replacement of the electrolytes and the fluid lost in the stools The exact defect of the disease is not fully known

SUMMARY

A case of congenital chloride diarrhoea (CCD) is described A short review is given of the clinical manifestations and pathogenesis of the disease and some problems concerning the treatment of the patient are discussed

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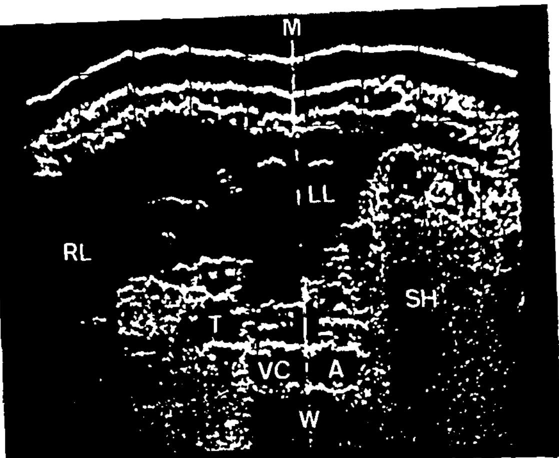


Fig 4 Ultrasonic scanning (Dr G Engelhardt D partment of Medicine University of Basel) The transversal ultrasonic tomography shows the liver (*RL LL*) the vena cava (*VC*) aorta (*A*) and the gastric shadow (*SH*) In the area of the pancreas an abnormal zone free of echo (*T*) corresponding to a pancreatic tumour

lesions by virtue of echo reflection from interfaces of tissues (2)

On surgery a round dark red tumour 1 5 cm in diameter was found in the pancreas at the junction of the head and the body Histology showed a mixed A and B islet cell adenoma with amyloid stroma (Fig 5) Tumour tissue was sent to Professor Gregory in Liverpool for gastrin extraction The activity of this extract was measured by Dr Halter (Department of Medicine University of Berne) by a modified perfused rat stomach preparation The injection of tumour extract resulted in a significant rise of secreted acid increasing the conductivity of the perfusate which started immediately at the end of the intravenous application An islet cell adenoma was thus diagnosed consisting of two cell populations and producing gastrin in addition to insulin (so-called insulinoma gastrinoma) The immediate postoperative course was uneventful Plasma insulin values fell promptly to normal No further episodes of hypoglycemia have been recorded

DISCUSSION

The most important diagnostic tests in a suspected insulinoma are measurements of blood sugar and plasma insulin during fasting and after administration of tolbutamide Tolbutamide often produces a dramatic rise in plasma insulin levels when an insulinoma is present (3) The near normal levels of IRI during the first tolbutamide test in our patient confirms similar observations in other reports and stresses the necessity of repeated testing (3 4) Furthermore multiple estimations of fasting plasma insulin may be required before a raised level is demonstrated

To our knowledge this is the first reported

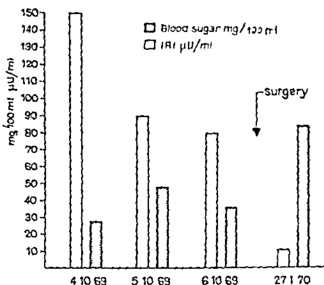


Fig 2 Insulin and fasting blood sugar before and after operation excessive plasma insulin levels in the presence of hypoglycemia before operation normal values after surgery (IRI-determinations Dr G Zahnd Department of Medicine University of Geneva)

epinephrine Fructose and galactose tolerance tests gave normal results Growth hormone and cortisol response to insulin induced hypoglycemia as well as the ACTH test showed an intact hypothalamopituitary adrenal system Functional hyperinsulinism has been excluded by a normal leucine tolerance test Insulinoma was suspected on the basis of the severity of the hypoglycemia as well as the lack of other potential causes in this age group such as liver disease, pituitary or adrenal disorders and diabetes

Diagnosis of organic hyperinsulinism

The diagnosis of organic hyperinsulinism has been proved by simultaneous determinations of fasting blood sugar and plasma insulin and by the tolbutamide test In the presence of hypoglycemia fasting plasma insulin levels were excessive and ranged from 80 to 150 micro-units (μU) of immunoreactive insulin (IRI) per ml (Fig 2)

Tolbutamide test (Fig 3 *a* *b*) It is important to note that the first tolbutamide test did not lead to the diagnosis Although the blood sugar was very low only the 30 minute value of IRI was slightly increased to 68 μU/ml On repeated testing 5 and 12 months later however IRI rose to definite pathological levels of 109 and 365 μU/ml respectively with a consecutive drop of blood sugar to hypoglycemic values

Localisation of the tumour

An attempt to localize the tumour by selective angiography of the celiac and superior mesenteric artery was unsuccessful The scintigram of liver and pancreas using 250 μCi selenium 75 methionine showed a slight reduction of the activity at the junction of the pancreatic head and body suggesting a tumour in this area The definitive diagnosis and localisation of a tumour was confirmed by an echographic (ultrasound) scanning of the upper abdominal region (Fig 4) This method allows cross sectional depiction of organs and

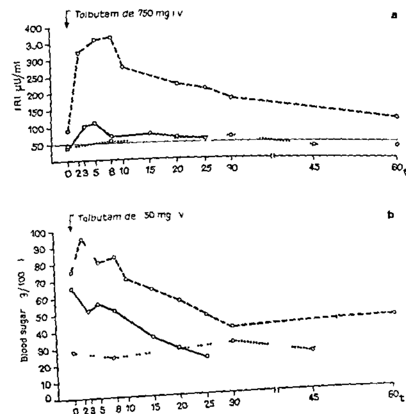


Fig 3 (*a*) IRI response to intravenous tolbutamide (*b*) Blood sugar response to intravenous tolbutamide O O 30 10 68 O—O 17 4 69 O--O 7 10 69

Acta Pædiat Scand 61 623–625 1972

CASE REPORT

FANCONI S ANEMIA IN A NEONATE

JUAN J GERSHANIK, SAMUEL K MORGAN and RICHARD AKERS

From the Department of Pediatrics Medical University of South Carolina Charleston South Carolina USA

In the 44 years since Fanconi s original de scription (2) in three male siblings of a lethal familial anemia associated with microcephaly brown skin pigmentation hypogonadism ex aggerated reflexes and strabismus over 150 cas s have been added to the world literature A broad range of embryonic developmental defects have been described in Fanconi s ane mia (4) This is a report of a case which demonstrates a singular combination of con genital anomalies with the early onset of hematologic manifestations and raises certain questions regarding variants of Fanconi s ane mia

CASE REPORT

This Negro male child was born at term to a hyper tensive 41 year old gravida IX para VIII abortus I woman No medications were taken except for an *iron preparation* There was no radiation expo ure and no maternal infection Six children are living and one died of drowning at age 16 years Fetal move ments were poorer than in previous pregnancies She was delivered by Cesarean section followed by hyster ectomy for sterilization Polyhydramnios was present The Apgar score was seven at 1 min Respiration was well established at 5 min Birth weight was 1 950 g length 49 cm and head circumference 33 cm The child was considered small for gestational age The patient had absence of the radii and thumbs and an imperforate anus Secretions drained from the mouth A brownish hyperpigmented area was observed in the lumbar region Difficulty was noted in passing a nasogastric tube

Roentgen examinations revealed a blind upper esophageal pouch extending to T vertebra Films of the upper extremities revealed bowing ulnas and ab sent radii and thumbs (Fig I)

A suction tube was placed in the proximal eso phageal pouch Definitive surgery was postponed tem porarily to permit a search for lethal cardiovascular or renal anomalies

A generalized petechial eruption appeared 24 hours after birth The platelet count was 6 000/mm³ WBC 8 300/mm with normal differential for age hemo globin 14 6 g/100 ml and hematocrit 43 volumes % Platelet counts subsequently varied between 6 000/ mm and 22 000/mm A bone marrow aspirate demonstrated hypocellularity erythroid hyperplasia and the absence of megakaryocytes There was an in crease in histiocytes Attempts at chromosomal cul ture from peripheral blood and bone marrow were un successful Umbilical vein catheterization was per formed to permit hyperalimentation feeding The child had a sudden cardiorespiratory arrest and died on the 8th day of life

Chromosome studies and hemo rams on both parents were normal X rays of the forearms and hemograms on the patient s four youngest siblings were normal

AUTOPSY FINDINGS

Scattered petechiae were present over the trunk The thyroid and thymus were normal The lunes showed minimal pneumonitis The heart was normal The superior portion of the esophagus ended in a blind pouch 3 cm below the pharynx The distal portion was connected to the trachea at its bifurcation point The stomach and lower gastrointestinal tract were nor mal throughout except for the atretic anal region The rectum ended in a blind pouch 1 / cm from the peri neum The liver gall bladder and pancreas were nor mal An accessory spleen was found in the mesentery A single left pelvic kidney was present Post mortem retrograde pyelography showed its major calyces to fill normally This kidney drained into a normal bladder The right kidney was absent Post mortem

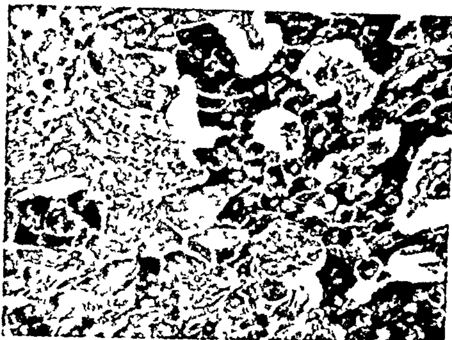


Fig 5 Ribbons of A_1 cells with strongly argyrophilic cytoplasm (dark cell clusters) between ribbons of B cells Hellerstrom Hellman s silverimpregnation 440 ×

case where a pancreatic tumour has been localized by ultrasonic scanning in a child

SUMMARY

An 11 $^{10}/_{12}$ year old boy with spontaneous hypoglycemia especially before meals since the age of 10 $^{3}/_{4}$ years is reported Epinephrine and glucagon tests as well as fructose and galactose tolerance tests were normal Insulin tolerance and ACTH tests showed an intact hypothalamo-pituitary adrenal axis Insulin measurements in fasting and tolbutamide test suggested an organic hyperinsulinism Diagnosis and localisation of a tumour at the junction of the pancreatic head and body using ultrasonic scanning was made Histological diagnosis a mixed A_1 and B islet cell adenoma with amyloid stroma (so called insulinoma gastrinoma) An islet cell adenoma was thus diagnosed consisting of two cell populations and producing gastrin in addition to insulin

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Fig 1 X ray of left forearm and hand Note absence of radius and thumbs and bowing of ulna

contrast roentgenographic studies did not reveal a rectovesical fistula Both testes were present in the scrotum but were hypoplastic The adrenals were normal in size though located ectopically The right adrenal was found near the anterior margin of the right lobe of the liver just adjacent to the diaphragm The left adrenal was found adherent to the sigmoid colon Bone marrow sections showed absence of megakaryocytes and erythroid hyperplasia Subarachnoid hemorrhage was present Stenosis of the aqueduct of Sylvius was present producing mild hydrocephalus

COMMENT

This infant had most of the common clinical and pathological findings described in Fanconi s anemia (4) Some of the unusual features, such as hydrocephalus (9) and tracheo oesophageal fistula (7) have been occasionally reported in isolated instances The patient s ethnic background is important Dawson (1) and Juhl et al (7) comment on the absence of racial preponderance However, Fanconi s anemia is rarely seen in Negro patients (10 12)

Other established syndromes which combine congenital skeletal abnormalities and hematologic findings were considered in this patient Because the early onset of the hematologic abnormalities and the selective affectation of the megakaryocytic series, a differential diagnosis of congenital hypoplastic thrombocytopenia (5 11) or thrombocytopenia with absent radii (6) was considered Fanconi (3) has emphasized that the range of onset of hematologic manifestations extends from the neonatal period to the middle of the second decade and it is recognized that the initial hematological abnormality can include amegakaryocytic thrombocytopenia (7 8) The low birth weight in this male infant the brownish hyperpigmentation of the skin the absent thumbs and the broad spectrum of congenital malformations make us believe that this patient qualifies as an instance of Fanconi s anemia Some overlapping features of Fanconi s anemia and congenital hypoplastic thrombocytopenia or thrombocytopenia with absent radii in this infant cannot go unrecognized

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S Vaage & L Efskind (Oslo Norway) *Congenital oesophageal atresia a follow up study*

Follow up of 36 patients operated on between 1948 and 1961 at Surgical Department A Rikshospitalet Oslo included 3 patients with tracheo-oesophageal fistula 2 with oesophageal atresia without fistula and 31 with both atresia and fistula

After 10 to 23 years 23 patients are now asymptomatic 6 have dysphagia which is only pronounced after eating rapidly and 7 suffer from severe dysphagia even when eating at normal speed

X ray examination of the oesophagus revealed that the anastomosis was either 1) without stenosis 2) moderately stenotic or 3) severely stenotic with the diameter reduced by more than 50 % Comparison of the complaints with the morphological state of the anastomosis showed that 20 of the asymptomatic patients have normal anastomosis or moderate stenosis the 6 patients with moderate complaints have moderate stenosis while of the 7 with severe complaints 1 has a normal anastomosis 2 moderate stenosis and 4 severe stenosis In the patient with a normal anastomosis the severe dysphagia may be due to a psychomotor disorder Severe dysphagia results when the anastomosis is severely stenosed (by more than 70) When stenosis is moderate on the other hand the situation varies 40 of patients are without symptoms but the others have dysphagia which is moderate in 40 % and severe in 20

The operation did not restore normal peristalsis In the proximal oesophageal segment normal peristalsis occurred but did not continue beyond the anastomosis In the lower oesophageal segment the peristalsis started from 1–2 cm to 10 cm above the cardia presumably in response to the oesophageal distension but bore no actual relation to the swallowing mechanism These abnormal spontaneous peristaltic movements did not necessarily result in propulsion of the pabulum

Peristalsis occurred in the inferior segments in about 60% of the patients without symptoms or with moderate complaints but only in 17% of those with severe symptoms

Function was evaluated manometrically with an Elema Mingograph 81 In the patients in whom oesophageal surgery had been performed normal motor function was found in the upper sphincter and the short proximal oesophageal segment In the intermediate segment a characteristic tonic pressure wave was observed No migration of the pressure wave was observed although an increase in pressure throughout the oesophagus was seen as in achalasia In the lower segment bizarre pressure waves occurred irregularly and occasionally did not cause propulsive migration However a normal lower sphincter was present in several cases

No definite conclusions could be drawn about the extent to which the dysphagia was correlated with the function of the oesophagus An upper sphincter was found in 8 of those who were asymptomatic in 3 with moderate symptoms and in 1 with severe symptoms The motor activity in the lower oesophageal segment seemed to be unrelated to the severity of the dysphagia

H Sommerschild F Langmark & K Maurseth (Oslo Norway) *Congenital hepatic fibrosis*

The first 2 cases of congenital hepatic fibrosis diagnosed in Norway were presented 1) A 9 year old boy with an uneventful case history in whom an enlarged liver was found incidentally (diagnosis confirmed by liver biopsy) Liver function tests were normal Portal venous pressure was normal Intravenous pylography (IVP) showed enlarged kidneys with normal function and without any indication of cystic disease or medullary sponge kidney No oesophageal varices were detectable The child is doing well 2) A 14 year-old girl who first had haematemesis at 10 years of age Portal hypertension and oesophageal varices were demonstrated The portal vein was patent

Acta Pædiat Scand 61 626–628, 1972

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS

Seventh Meeting, May 13–15 1971, Uppsala Sweden

I Panel discussion *Operations for diversion of urinary flow Why how other possibilities?*
Chairman N O Ericsson
On the panel R Bjordal A Nergårdh K Parkkulainen, A Reuterskiold B Henrikson and G R Wallgren

II Panel discussion *Water salt and calories in neonatal surgery*
Chairman G Grotte
On the panel H C Borresen S Froberg L Hambraeus O Knutrud R Olegård B Persson A Wretlind S Jacobson and E Vinnars

FREE PAPERS

Bjorn Henrikson & Olof Brodin (Gothenburg Sweden) *Urinary diversion 1950–70*

At the Surgical Department of the Children s Hospital Gothenburg urinary diversion was performed in 34 patients during the period 1950–70

The diagnosis was myelomeningocele in 12 cases extrophy of the bladder in 16 valve in the urethra in 3 and myosarcoma of the bladder, sclerosis of the bladder neck and bilateral stenosis of the ureters in one case each

Most of the patients were operated upon between the ages of 0 and 2 years The following types of operation were performed Cutaneous ureterostomy in 17 cases uretero colostomy in 14 and uretero ileo cutanostomy in 3 cases

Six patients were lost 2 with myelomeningocele 3 with estrophy of the bladder and the one with myosarcoma The causes of death were Ileus in 3 cases and postoperative shock renal insufficiency and metastases in one case each

In 6 cases one kidney was destroyed and had to be removed Cutaneous ureterostomy was performed in 4 of these cases uretero colostomy in one and uretero ileo cutanostomy in one

After the diversion urinary infections were seen in 7/12 with myelomeningocele in 6/16 with extrophy of the bladder and in 4/6 of the other patients In 16 cases stenosis of the ureters occurred postoperatively Hydronephrosis due to the kinking of the ureter around the vertebral column was often recognized in the kidney contralateral to the site of the cutaneous ureterostomy

I Louhimo & M Pasila (Helsinki Finland) *Accessory lung Report of nine operated cases*

During the period 1961–1969 9 patients with extralobar sequestration or accessory lung have been operated on at the Children s Hospital University of Helsinki The age of the patients ranged from 3 months to 12 years, 5 were boys and 4 girls The accessory lung was on the left side in 7 and on the right side in 2 cases Only 1 patient represented the lower accessory lobe variety with diaphragmatic malformation all the other aberrant lungs were in the upper mediastinum The arterial and venous supply and bronchial architecture of the accessory lungs were described as well as the typical chest X ray findings in cases of superior accessory lung



H A WEIJERS IN MEMORIAM

H A Weijers who died in June 1972 was born in Tilburg The Netherlands in 1914 He got his medical education at the State University of Utrecht during 1935–1942 From 1942–1946 he got his pediatric training at the Wilhelmina Children s Hospital under Prof ten Bokkel Huinink After specialization he opened a private practice in Utrecht but kept a close connection in research with the Wilhelmina Children s Hospital and the Central Institute for Food and Nutrition Research In 1957 he was appointed Reader in Pediatric Clinical Nutrition and in March 1963 he was inaugurated Professor of Pediatrics at the State University in Utrecht and at the same time appointed Medical Director of the Wilhelmina Children s Hospital Weijers has for many years been a member of the advisory board of Acta Paediatrica Scandinavica and has made frequent contributions to this journal

Weijers thesis was entitled Fat absorption in normal and abnormal infants and children with special reference to celiac disease and was published in 1950 The year before he had together with van de Kamer and ten

and normal by splenoportography Liver function tests were normal Splenectomy and splenorenal anastomosis were carried out Liver biopsy revealed biliary cirrhosis During a four-year follow up, persistent oesophageal varices were demonstrable In spite on this the patient was doing well At 14 she had a second haematemesis of moderate quantity A review of the liver biopsy specimens supported the diagnosis of congenital hepatic fibrosis (CHF) IVP showed enlarged kidneys with cystic disease, but both renal and liver function were normal A portacaval shunt has now been done the postoperative course was unremarkable

S Bergdahl C Hugosson, T Lauren and S Soderlund (Stockholm Sweden) *Atypical intussusceptions*

From January 1956 to May 1971 253 patients with intussusceptions were seen at the Children s Clinic of Saint Gorans Hospital and the former Kronprinsessan Lovisa s Children's Hospital

The age of the patients ranged from 3 days to 10 years 150 being under the age of 2 years

The intussusceptions were divided into the following types ileo ileal caeco colic and ileo colic

Sixty-two per cent of the patients were treated with barium enema reduction The remaining 38% were operated on after diagnostic barium enema only or failed attempts to reduce the intussusception with a barium enema

The following cases were regarded as atypical

1 Spontaneous reduction found at laparotomy performed after failure to reduce with a barium enema 27 patients In all these cases the roentgen findings were typical of intussusception and in most cases the diagnosis was confirmed at operation by the presence of swelling of the bowel distal to a segmental constriction

2 Chronic course 2 patients, one with lymphosarcoma and one with Meckel s diverticulum

3 Recurrence 20 patients

4 Pathological lesion found as leading point 15 patients

The leading points' were Meckel s diverticulum 6 cases, anaphylactoid purpura 4 enterocystoma 3, lymphosarcoma 2

Two patients died one from spread of the lymphosarcoma and one from bowel necrosis after operative reduction

It may be possible to increase the number of successful barium enema reductions by trying another reduction after the patient has been sedated for operation

R Bjordal (Oslo Norway) *Gastroschisis*

Over a 2 ½ year period 9 cases of gastroschisis were diagnosed and treated surgically Six patients survived and are doing well Three patients died of septic *Candida albicans* infection probably as a consequence of thrombophlebitis due to periphrenal vein infusion and of antibiotics In every case the intestines were definitely shorter than normal In 5 patients primary closure of the total abdominal wall was possible (1 died) and in the other 4 operations the silastic bag principle was used The importance of adequate total parenteral feeding infused through scalp veins and of not giving antibiotic prophylaxis is stressed

J Gierup & N O Ericsson (Stockholm Sweden) *Congenital bladder neck obstruction—urodynamic aspects*

A recent series of 15 boys with congenital bladder neck obstruction is presented In 12 simultaneous measurements of intravesical pressure and urinary flow were made The urodynamic findings deviate significantly from normal Although the roentgenological picture is relatively uniform the variety of urodynamic patterns suggest several different aetiological mechanisms

G R Wallgren

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Helsinki
Finland

Acta Pædiat Scand 61 629–631 1972



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Bokkel Huinink, published a new method for the determination of fecal fat, a method which is still used today at most children's hospitals in the world In a critical evaluation of various methods at that time used for malabsorption studies he concluded that the most reliable method was measurement of the coefficient of fat absorption In the early 1950's he demonstrated—together with his close co workers Dicke and van de Kamer—in a series of brilliant investigations that removal of wheat from the diet caused the symptoms and signs of celiac disease to disappear, whereas reintroduction of the same foodstuff caused recurrence At that time the current opinion was that all starch containing foodstuffs (with the exception of bananas) were injurious to the celiac patient and must be avoided The Dutch research group now showed that it was not the starch content of the diet but the kind of food which was the important factor They could then prove that it was the protein of wheat flour gluten that was responsible for the noxious effect when gluten was added to the diet of a celiac patient the signs and symptoms of the disease reappeared The harmful action of gluten was shown to be chiefly bound to the gliadin fraction In agreement with clinical experience foodstuffs having a high ratio amid nitrogen non amid nitrogen were found especially deleterious Already 1935 Holt had shown that in normal infants the rate of absorption of fat was inversely proportional to the degree of saturation of the fatty acids of the dietary fat although the difference between different acids was not very large This difference was found to be more accentuated in celiac disease, Weijers and van de Kamer thus showed that the absorption of fat composed of oleic acid was only slightly decreased while saturated fat caused a marked steatorrhoea This was later confirmed by others showing that fat containing polyunsaturated fatty acids such as linoleic acids is about normally absorbed in celiac disease The explanation for this is still unknown, it is, however, quite clear that it cannot be due to the general reduction of the surface area in celiac disease caused by the villi atrofy Weijers postulated some disturbances of the fat metabolism It should be especially mentioned that the Dutch group published their study on celiac disease in a series of 7 papers in Acta Paediatrica

In 1960 Weijers and his co workers started a series of investigations related to intestinal intolerance of sugar They could show that in certain patients severe diarrhoea was due to an inability to split sucrose and/or maltose this was in turn caused by a deficiency of the corresponding disaccharidases Loading with the offending sugar caused in these patients a marked fecal excretion of certain organic acids, chiefly lactic acid He was already at that time aware that this type of disturbance existed, not only as a congenital disorder but also—and maybe more commonly—as secondary deficiencies in different diseases of the intestinal tract Each process by which the cells of the intestinal wall are damaged either anatomically or functionally may cause a decrease of the enzymatic function Weijers discussed also more extensively the formation of lactic acid and other organic acids in diarrhoea due to carbohydrate intolerance introducing or reemphazising the concept of fermentative diarrhoea

During recent years Weijers paid special attention to the study of protein absorption and the relation of intestinal dysbacteriosis to so called putrefactive diarrhoea The role of dipeptidases in the pathogenesis of celiac disease was another research branch His list of publications comprises besides investigations on malabsorption syndromes various other papers e g studies on resistent rickets

Although not a friend of paragraphs restrictions and regulations Weijers was a very clever organizer He was able to gather around him very skillful co workers in different branches of pediatrics in nutrition and in biochemistry His main research interest was the field of pediatric gastroenterology and clinical nutrition and he was one of the

initiators of the European Society for Pediatric Gastroenterology and became its first president At that time he was well known over the world as lecturer and as participator in several symposia

The primary goal for Weijers was to give each child good care in an atmosphere specially suited to children He always had interest in the social wellfare of his small patients A generous attitude not only towards his patients but also towards his friends was characteristic of him It was a privilege to visit him at home with his family to meet the general harmony there was one of the most subtle experiences one could have However serious in his scientific work he had a genuine and often boisterous humour and could also in this way help many of his co-workers and friends to master their problems

Dolf' Weijers will be remembered for a long time not only because of his contribution to science but also because of his outstanding personality

Bertil Lindquist

Acta Pædiat Scand 61 632–642 1972

NEONATAL CHOLESTASIS IN ALPHA-1-ANTITRYPSIN DEFICIENT CHILDREN

Clinical Genetic Histological and Immunohistochemical Findings

Ø AAGENÆS A MATLARY K ELGJO E MUNTHE and M FAGERHOL

From the University Hospital Departments of Pediatrics Pathology and Rheumatology and the Ullevål Hospital Blood Bank Oslo Norway

Since the report of Laurell & Eriksson in 1963 (7) the alpha-1 antitrypsin deficient state (Pi type ZZ) has been connected with pulmonary emphysema in early adulthood In 1969 Sharp et al (10) showed that some of his patients with juvenile cirrhosis also were homozygote for the alpha-1 antitrypsin deficiency gene Pi^{Z} A few similar cases have also been reported from New York (4) and from Boston (6)

In these materials about 50% of the children with cirrhosis had had a cholestatic phase in early infancy

Recently Sharp (11) has found that the liver cells of these children with cirrhosis contain an increased amount of alpha 1-antitrypsin, studied by fluorescent tagged antibody against alpha-1 antitrypsin

The following paper stresses the clinical genetic histological and immunohistochemical findings in 5 children with neonatal cholestasis and alpha-1 antitrypsin deficiency It also reports histological and immunohistochemical findings in one patient with emphysema and one individual without clinical lung- or liver disease both with alpha 1 antitrypsin deficiency and histochemical studies on the liver in three MZ individuals

MATERIAL

As neonatal cholestasis was frequent in the reported patients with alpha 1 antitrypsin deficiency and cirrhosis we searched our records from recent years for patients with neonatal cholestasis of unknown origin Where the protein electrophoresis showed a low alpha 1 globulin peak a specific examination of the alpha 1 antitrypsin and the Pi type was performed (2) The following 5 patients were found to have alpha 1 antitrypsin deficiency with Pi type ZZ

Case 1 F Ø male b 1957

No 2 of 2 children B w 3 500 g Jaundiced at 2 weeks of age with light coloured stools and bleeding from the umbilicus No information on duration of cholestasis Liver cirrhosis recognized at age 5 with portal hypertension and oesophageal varices On examination at age 12 his bromsulfthalein retention was 15% galactose load was borderline normal serum albumin was 3 2 g/100 ml and γ globulin 2 6 g/100 ml Good clinical condition and no bleeding from the oesophageal varices at age 14 Pi typing showed Pi type ZZ alpha 1 antitrypsin conc 13 % of normal The parents had Pi type MZ with alpha 1 antitrypsin conc 60–70% of normal

Case 2 J I J male b 1968

First child b w 2 980 g at term At 16 days umbilical bleeding and slight jaundice Examination showed low values for vitamin K dependent coagulation factors with improvement after vitamin K The degree of jaundice increased (max bilirubin 8 4 mg/100 ml) and the stools were light coloured Liver biopsy showed fibrosis proliferation of the bile ducts cellular infiltration cholestasis and parenchymatous degeneration The cholestatic phase lasted for about 6 months Since then the clinical condition has been satisfactory but transaminases and alkaline phosphatase are permanently increased Pi type ZZ, alpha 1 antitrypsin conc 18 % of normal The parents both had Pi type MZ with alpha 1 antitrypsin conc 68 and 78% of normal

Case 3 M B female b 1968

No 3 of 3 normal birth 10 days after term b w 2 850 g Jaundice from the first weeks stools light

coloured Poor weight gain Admitted at age 2 / months jaundiced with hepatosplenomegaly Lab examinations showed moderate increase in trans aminases alkaline phosphatases and lipids Se bili rubin 5 9 mg/100 ml → 0 9 mg/100 ml Expl laparotomy showed normal biliary tract Biopsy of the liver showed proliferation of the connective tissue and bile ducts cellular infiltration and parenchyma tous degeneration Cholestasis cirrhosis?

Clinical cholestasis disappeared at age 3 months and the patient has developed normally Transamin ases and alkaline phosphatases till moderately in creased Pi type ZZ, alpha 1 antitrypsin conc 33 % of normal Parents genotype MZ, alpha 1 antitrypsin conc 59 and 88% of normal Two siblings genotype ZZ, without obviou clinical signs of disea e

Case 4 C E female b 1970

No 2 of 2 normal birth Jaundice from before 4 weeks light stools and dark urine Se bilirubin 15 5 mg 100 ml (66 conjugated) at age 5 weeks Trans aminases alk phosphatase cholesterol and triglyc erides all moderately increased Radioactive Rose Bengal test showed 12 excretion in feces in 2 days—probably intrahepatic cholestasis Explorative laparotomy showed normal extrahepatic bile ducts Biopsy showed biliary stasis with proliferation of bile ducts fibrosis and cellular infiltration D Cholestasis biliary cirrhosis? On reexamination at age 18 months the child still showed signs of cholestasis slight jaundice pruritus xanthomatosis and growth re tardation Lab tests still showed increased trans aminases alk phosphatases cholesterol and tri glycerides Pi type ZZ Alpha 1 antitrypsin conc 41% Parents both Pi type MZ alpha 1 antitrypsin conc 62 and 75 of normal

Case 5 A A male b 1970

No 2 of 2 normal birth b w 3 200 Jaundice from before 2 weeks, stools light coloured urine dark. At admittance (4 weeks old) slight jaundice and hepatosplenomegaly Explorative laparotomy showed normal extrahepatic bile tree Biopsy showed biliary stasis, proliferation of bile ducts moderate fibrosis severe cellular infiltration D Chronic infection cholestasis fibrosis

Because of rupture the child had to be resutured 3 days later 3 days after the last operation the patient developed pneumonia and died Lab examinations showed moderately increased transaminases and alk phosphatase Pi type ZZ alpha 1 antitrypsin conc 16% of normal Parents Pi type MZ alpha 1 antitrypsin conc 87 and 82% of normal

In summary these 5 patients all had neonatal cholestasis which usually seems to have sub sided at age 1 year In 2 patients the clinical picture was indistinguishable from biliary at resia Two patients were referred because of umbilical bleeding In the following years the patients had relatively few symptoms for long periods but liver cirrhosis with portal hyper tension developed

In addition to the studies on the ZZ patients with neonatal cholestasis liver biopsies fo immunohistochemical studies were also per formed on 2 adult individuals with Pi type ZZ but no clinical liver disease on 1 child and 2 adults (the mother of C E. and the father of J I J) with Pi type MZ and in children with intra and extra hepatic cholestasis and Pi type MM

Clinical data on these individuals were as follows

Case 6 G K. male b 19 6

Mother died from jaundice of unknown type From age 20 coughing from age 40 dyspnoea Now severe emphysema Pi type ZZ, alpha 1 antitrypsin conc 20 of normal No signs of liver di ease normal se transaminases Aspiration biopsy from liver performed Only material for immunohistochemical studies

Case 7 I M O female b 1941

Father (Pi type MZ 58% of normal) emphysema from age 60 Patient diab mell from age 14 No lung nor liver symptoms but moderate increa e in OCT (160 U) and moderate decrease in se albumin (2 8 g/100 ml) Normal transaminases Pi type ZZ, alpha 1 antitrypsin conc 45% of normal

Case 8 V E. female b 1944

(Mother of C E case 4) No signs of diseae normal liver functions Pi type MZ, alpha 1 antitrypsin conc 75% of normal

Case 9 L J male b 1933

(Father of J I J case 2) No signs of disease Nor mal liver functions Pi type MZ, alpha 1 antitrypsin conc 68% of normal

Case 10 E J male b 1970

Admitted because of stridor and some hepatomegaly Normal liver findings Pi type MZ, alpha 1 anti trypsin conc 50% of normal

Case 11 K R female b 1970

Brother neonatal cholestasis with giant cell trans formation died with hepatoma Patient neonatal cholestasis with giant cell transformation improved during first year but still pronounced liver findings at 1 year of age Pi type MM

Case 12 E. R male b 1971

Admitted because of cholestasis Explorative laparotomy showed atresia of the biliary tree Pi type MM

Table 1 *Comparison of alpha 1 antitrypsin content in serum and in liver biopsies*

Case	Pi type	of normal serum conc	Highest dilution of conjugate giving positive tissue reaction	Approx positive hepato cytes	Extra cellular deposits
2 J I J	ZZ	18	1 512	40–60	+ + +
4 C E	ZZ	41	1 512[b]	40–60	+ + +
6 G K[a]	ZZ	20	1 512	20–40	+
7 I M O	ZZ	45	1 512	20–40	+ + +
8 V E	MZ	75	1 128	1–5	0
9 L J	MZ	68	1 256	1–5	0
10 E J	MZ	50	—	0	0
11 K R	MM	160	—	0	0
12 E R	MM	140	—	0	0

[a] Liver aspirate
[b] The same titre was obtained before and after the patient had been given several infusions of plasma containing alpha 1 antitrypsin
Amounts of extracellular deposits were graded from + to + + + No such deposits were recorded as 0

Histological Examinations

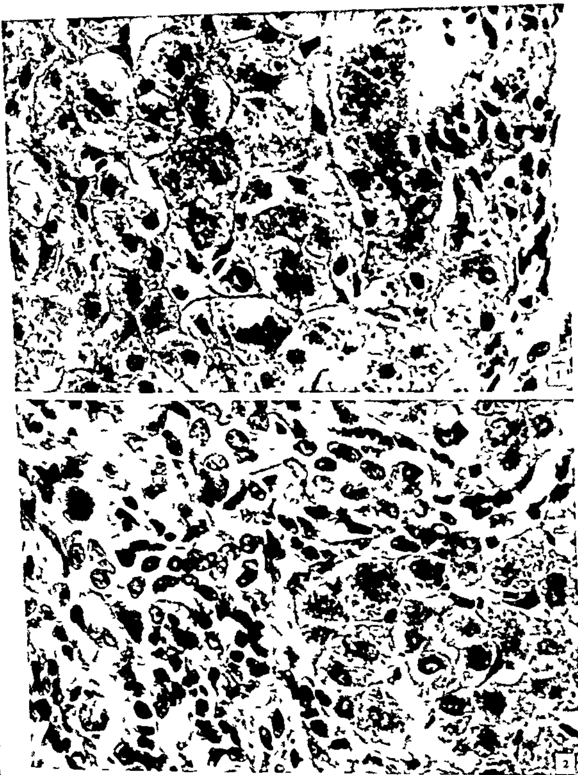
As is evident from the primary description the biopsies from the different patients with Pi type ZZ and cholestasis showed a very varied histological pattern Cholestasis was a prominent feature in all of them In addition all biopsies revealed portal fibrosis portal infiltration of lymphocytes plasma cells and eosinophil granulocytes bile duct proliferation and piecemeal necrosis of the parenchymal cells Thus a chronic aggressive hepatitis was suspected in some cases while a primary biliary cirrhosis appeared more probable in others

On re examination of the specimens we noticed that all biopsies had one distinctive feature in common In all biopsies we found a finely granular greyish brown pigment located especially in the parenchymal cells surrounding the portal tracts (Figs 1 and 2) Some scattered parenchymal cells with the same pigment were seen even at some distance from the portal tracts This pigment was more finely granular than haemosiderin and the colour was more dull It could also easily be distinguished from the bile pigment that was present in some cells Several of the parenchymal cells containing this pigment were larger than the surrounding cells Although the Kupffer cells were enlarged we could not see any pigment in these cells The pigment did not change colour when the specimens were stained for iron (according to Perl) or with PAS (periodic acid Schiff)

This peculiar pigment was seen even in an adult whose antitrypsin deficiency was discovered incidentally during hospitalisation for another disease (case 7) In the liver biopsy from this patient the pigment was found only in some scattered parencyhmal cells without predilection for the periportal areas The pigment was not seen in any of the MZ cases studied (case 8 9 and 10) or in the other cases with cholestasis and Pi-type MM (Cases 11 and 12)

Fig 1 In this high power microphotograph almost all parenchymal cells contain the typical brownish granules The granules are darker than hemosiderin and more finely granular Connective tissue from a portal tract is seen in the right part of the picture Bile in parenchymal cells and in bile canaliculi appears black (Init magn × 660)

Fig 2 The parenchymal cells lying adjacent to the portal connective tissue are partially filled with granular brown pigment In the portal connective tissue several degenerated parenchymal cells are seen Also there is extensive bile duct proliferation and diffuse infiltration of lymphocytes and some eosinophil granulocytes (Init magn × 660)


1
2

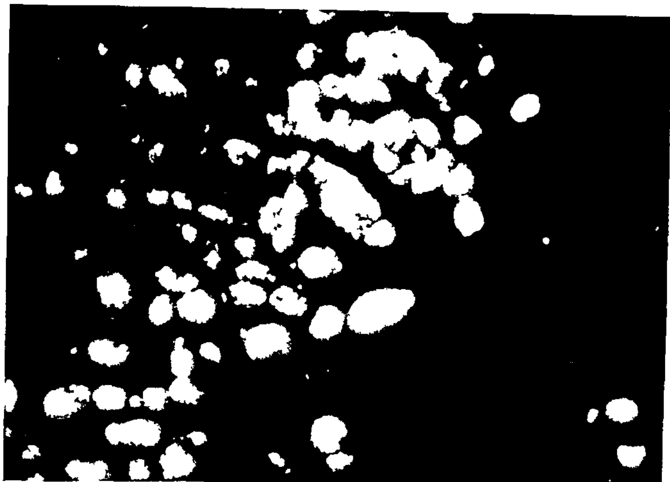
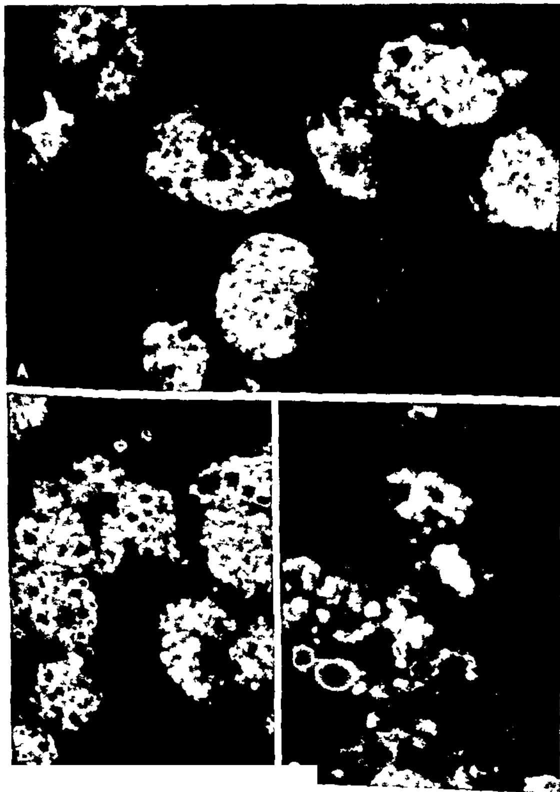


Fig 3 Frozen section from liver biopsy of ZZ homozygeous patient C E stained with FITC labelled anti alpha 1 antitrypsin The hepatocytes located adjacent to the unstained fibrotic areas are particularly strongly stained (×800)

Immunohistochemical and Immunological Examinations

Liver biopsies were embedded in Tissue tec OCT compound, quick frozen in dry ice/acetone-isopenthane and sections cut in a cryostat The various protein antigens were detected by direct immunofluorescence staining as previously described (9) Alpha 1 antitrypsin was identified by means of fluorescein isothiocyanate (FITC) labelled anti alpha 1 antitrypsin obtained from Behringwerke AB The conjugate was made specific by absorption with other human serum proteins and was purified by gel filtration to avoid unspecific fluorescence FITC labelled anti alpha 2 macroglobulin also was obtained from Behringwerke AB IgG antibodies against human IgG IgA IgM the complement component C3 and albumin were produced by rabbit immunization isolated labelled with FITC and used to detect the respective antigens (9) The results after staining with anti alpha 1 antitrypsin are shown in Table 1 All patients with Pi type ZZ had large amounts of alpha 1-antitrypsin in from 20-60% of their hepatocytes (Figs 3 and 4) The protein was usually concentrated in periportal liver cells or in cells adjacent to the fibrous areas in cirrhotic cases The amount of protein varied considerably from

Fig 4 (*A*) Liver section from ZZ homozygeous patient I M O with no liver disease stained for alpha 1 antitrypsin Several hepatocytes are positively stained but many hepatocytes are also negative (*B*) Liver section from ZZ homozygous patient J I J similarly stained showing alpha 1 antitrypsin in small cytoplasmatic vacuoles (*C*) Section from the same biopsy showing alpha 1 antitrypsin in larger probably extracellular deposits (× 1 250)


A

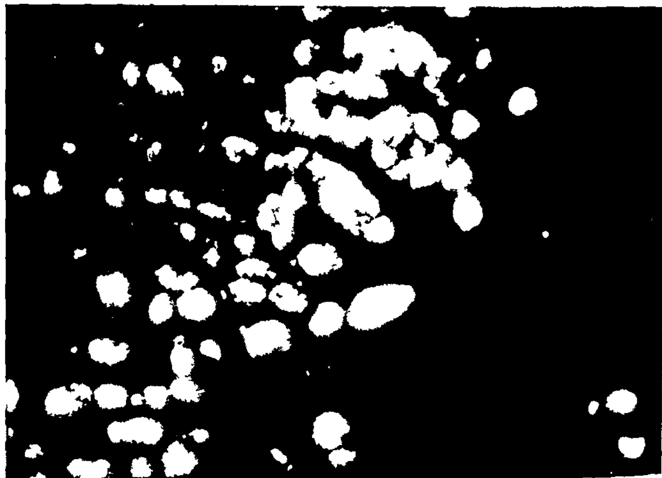


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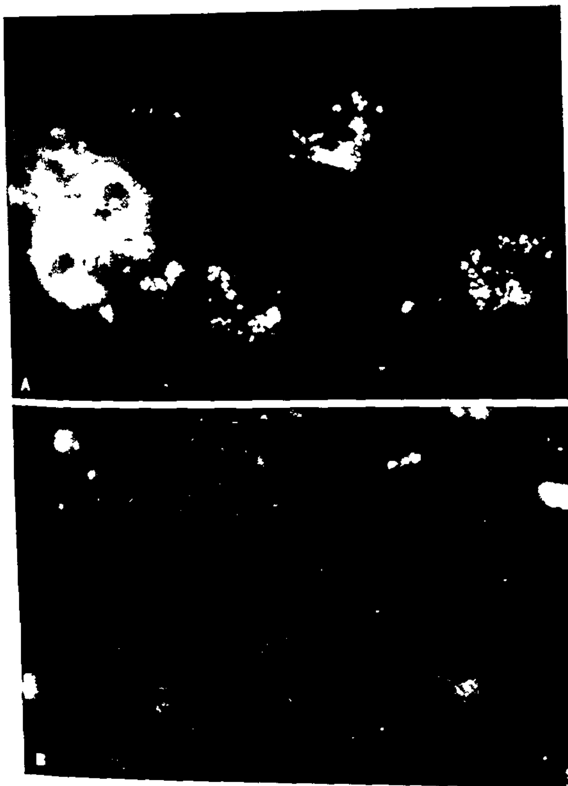


Fig 5 (*A*) Liver section from MZ heterozygeous patient L. J showing a cluster of two hepatocytes staining positively with anti alpha 1 antitrypsin (*B*) An area giving almost no positive staining in the same section Such areas could also be found in tissues from ZZ homozygeous patients (× 1 250)

one cell to another Some cells were stuffed with protein while others contained only a few granules The positive cells appeared often in clusters The protein often semed entrapped in small intracellular vesicles, and large amounts could in some cases be detected as extracellular amorphous deposits Even in positive tissues large areas showed no fluorescence (Fig 5 *B*) The percentage of cells with alpha 1 antitrypsin was at least ten times higher in ZZ than in MZ livers

A quantitative estimation of the amount of alpha 1 antitrypsin in the liver tissue was obtained by dilution of the standard conjugate (protein conc 1 mg/ml) until the tissue became negative The staining titre is recorded in the table The approximate percentage of hepatocytes detectable was recorded in each case The presence of tissue alpha 1 antitrypsin was dependent on the deficiency gene Z since no fluorescence was observed in the two MM livers There was however no correlation between the amount of liver alpha-1 antitrypsin and degree of damage Fibrotic areas were usually negative for alpha 1 antitrypsin

Two of the three biopsies from heterozygous MZ patients showed a few cell clusters or some dispersed single cells that stained positive for the anti enzyme (Fig 5 *A*) The amount of alpha-1 antitrypsin in these cells was lower than in the homozygous ZZ cases judged by the intensity of fluorescence and the highest dilution of conjugates giving a positive reaction The intracellular protein deposits were more finely granulated and fewer vesicles were observed Extracellular deposits were not found The third biopsy from a heterozygous patient (E J case 10) was negative None of the homozygous MM patients with normal plasma alpha-1 antitrypsin concentrations had detectable alpha 1 antitrypsin in their liver biopsies The tissue specimens were also stained to detect the various immunoglobulin classes C3 alpha 2 macroglobulin and albumin but none of these proteins were detectable in the hepatocytes Twenty liver biopsies, representing a wide spectrum of liver diseases from patients with normal alpha 1-globulins were also screened for alpha 1 antitrypsin but were negative Sera from the patients C E and I M O were examined for eventual presence of rheumatoid factors and antinuclear antibodies with negative results Immunological quantitation of their serum immunoglobulins showed some minor pathological variations but no characteristic pattern

In conclusion The immunohistochemical examination showed that the deposition of alpha-1-antitrypsin in the hepatocytes was correlated in each case to the degree of alpha 1-antitrypsin deficiency in the serum and dependent on the deficiency gene Z It was thus possible to differentiate between the ZZ the MZ and the MM individuals by means of immunofluorescence tissue examinations

DISCUSSION

Alpha-1 antitrypsin is a low molecular weight broad spectrum protease inhibitor It can inhibit many different proteolytic enzymes like trypsin, chymotrypsin, plasmin thrombin elastase collagenase hyaluronidase and proteases from leucocytes and microorganisms A large number of inherited variants of alpha 1 antitrypsin has been described and these constitute the Pi system (1) Patients with the Pi type ZZ have been found to have an increased frequency of emphysema and now also of liver disease in childhood

Ganrot et al (3) mentioned in their report on alpha 1 antitrypsin deficiency and emphysema that two of their patients died of liver cirrhosis

Sharp (11) showed with the same immunohistochemical technique as used in this material accumulation of alpha 1 antitrypsin in liver cells in children with cirrhosis and alpha 1 antitrypsin deficiency Sera from his patients have been tested in our laboratories and gave clear cut Pi ZZ patterns Electromicroscopical studies disclosed an amorphous material confined to the lumen of rough endoplasmic reticulum which was markedly dilated

Our own studies have shown that the ac-

two or three may develop early liver cirrhosis 6-7 emphysema in adult age and 1–2 no clinical symptoms

Obstructive liver disease in early infancy is not a frequent problem in pediatric departments, but it is on the other hand a very puzzling problem

Our limited data can only give a rough provisional estimate of the frequency of alpha 1 antitrypsin deficiency in children with cholestatic liver disease In our department we had 4 new cases of alpha 1 antitrypsin deficiency and cholestasis 12 patients with extra hepatic atresia 2 patients with familial giant cell transformation and 4 patients with other intrahepatic cholestasis of unknown cause during a period of about 36 months In addition 14 patients with septicemia had jaundice of more or less cholestatic type The relative frequency of the ZZ genotype in children with intrahepatic cholestasis without septicemia was therefore about 40

Since the clinical course in ZZ patients varies considerably additional factors genetic and/or environmental are probably necessary for the development of disease Very little is known about such factors So far the reports of liver disease and emphysema in the same family are rare As the liver disease in ZZ children is so newly recognized we could not expect any of their ZZ siblings yet to be in the emphysema age group We would expect that some siblings of the emphysema ZZ patients should have had liver disease in childhood This has so far not been reported but since only scanty histories of supposedly irrelevant conditions appear in most hospital records we do not believe any conclusions can be drawn from this

We must suggest that the additional factors predisposing to emphysema are different from those giving liver disease

Chronic irritation due to recurrent infections inhalation of fumes cigarette smoke or dust may result in increased liberation of proteolytic enzymes from leucocytes and macrophages With a relative lack of inhibitors in plasma such enzymes may damage the pulmonary capillaries and alveoli On the other hand no well formulated hypothesis has been advanced for the pathogenesis of the liver disease in ZZ individuals The alpha 1 antitrypsin in sera from ZZs is able to inhibit a series of proteolytic enzymes like the normal alpha 1 antitrypsin and to a degree reflected by the protein concentration It therefore seems unlikely that excessive proteolytic activity is the cause of damage in livers containing large amounts of a broad spectrum protease inhibitor The liver disease may rather be a storage disease in the sense that accumulation of alpha 1 antitrypsin (non specifically) is detrimental to vital cellular processes or alternatively that excess of this inhibitor interferes with important intracellular proteolytic enzymes Such enzymes may be involved in the detoxification of proteins and peptides arriving with the portal blood Against the storage theory stands the fact that ZZ patients without liver disease have the same amount of alpha 1-antitrypsin stored in their liver cells as the ZZ patients with liver cirrhosis

Lieberman et al (8) mention Au antigen as a possible additional factor for the liver disease As our patients were all Au antigen negative our findings do not support this theory The hypotheses are important to keep in mind when attempts at specific treatment are planned If the liver disease arises from the alpha 1 antitrypsin accumulation substitution therapy with human alpha 1 antitrypsin or synthetic protease inhibitors will have no effect One should rather explore the possibilities to stimulate or trigger the mechanisms involved in release of material from endoplasmic reticulum If successful such treatment may even provide enough alpha 1 antitrypsin to the plasma to protect the pulmonary tissue

SUMMARY

Five children with Pi type ZZ (alpha 1 antitrypsin deficiency) and liver disease two adult

cumulation of alpha 1-antitrypsin in the liver cells is not only found in ZZ patients with cirrhosis but in all ZZ-individuals studied, also in I M O without any clinical liver- or lung disease

In two of three examined livers from MZ persons alpha 1 antitrypsin was found in some liver cells by the immunohistochemical examinations The third MZ biopsy, which was negative was from a boy at age one year, while the other two MZ individuals were adults (parents of ZZ children with liver disease) One reason for the negative finding in this child may be that accumulation of alpha-1 antitrypsin in the liver cells has not yet reached a significant degree, or the biopsy may not have been representative

Our studies have shown that the ZZ patterns obtained when sera from ZZ children with intrahepatic cholestasis are tested by acid starch gel electrophoresis and antigen antibody crossed electrophoresis are indistinguishable from those obtained when sera from other ZZ individuals (apparently healthy children blood donors and emphysema patients) are tested Furthermore all the parents of the ZZ children with liver disease in this study had the expected MZ type when tested by the same methods We would not exclude however, that a minority of the children with this syndrome may have other Pi genotypes for instance SZ The possibility should also be kept in mind that some alpha 1 antitrypsin variants may not be released at a normal rate from the liver cells despite a normal electrophoretic mobility of the protein

It has recently been shown (5) that the loci for the inherited variants of alpha 1 antitrypsin (Pi) and IgG (Gm) are situated on the same chromosome Such linkage has been detected for Pi^Z as well as other alleles The accumulation of alpha 1 antitrypsin in the liver cells of ZZs is therefore most probably due to the synthesis of a structurally different protein because of a mutation at the locus for the alpha 1 antitrypsin s structural genes The liver cells may, in other words be unable to secrete abnormal proteins This is in accordance with the recent report (8) that even subjects homozygous for another variant (Pi^S) show a similar accumulation

Immunohistochemical studies in the two patients with giant cell cholestasis (case 11 K R) and extrahepatic atresia (case 12, E R) revealed no accumulation of alpha 1 antitrypsin in their liver cells

Clinical or subclinical liver affection seems to be frequent in the homozygote ZZ individuals Of the two adult ZZ individuals with out major liver disease, one had somewhat increased liver enzyme values in serum, and the one histologically examined (I M O) also showed some slight degree of liver fibrosis Of two other adult ZZ individuals that we have tested one showed increase in liver enzymes Although we have not obtained liver biopsies from ZZ-children without liver disease, the histological and laboratory findings in the adult ZZs support the view that this genotype is closely associated with liver disease

The greyish brown pigment found in the ZZ livers by ordinary histology is probably identical with the alpha 1-antitrypsin revealed by the immunofluorescence The lack of finding of this pigment with the ordinary histological methods in any of the MZ individuals is probably related to the lower amount of alpha 1 antitrypsin in their livers

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A Early cirrhosis of the liver with death before adult age Relative risk (of all ZZs) 20–30% ?

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The frequency of the ZZ state in Norway is about 0 2 per thousand (2) which gives about 12 ZZ individuals annually Of these

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KNUT LINCOLN GUNILLA LIDIN JANSON and JAN WINBERG

From the Departments of Clinical Bacteriology and Paediatrics University of Goteborg and the Department of Paediatrics at St Gorans Hospital Karolinska Institute Stockholm Sweden

There is a faecal and periurethral reservoir for bacteria causing urinary tract infections (1 4 9 11 15 16 18 19) The fact that several antimicrobial agents such as chloramphenicol ampicillin and the tetracyclines (3 5 6) sulphonamide (11 20) and cephalexin (8) may promote colonization of the bowel with resistant Gram negative organisms must be considered when treating urinary tract infections In an earlier study of children treated in hospital with sulphonamide (11) colonization of the bowel with multiple resistant *E coli* 04 preceded reinfections with organisms of the same serological type and resistance pattern It was concluded that an antibacterial agent ought to affect the faecal flora as little as possible In the present study sulphonamide and nitrofurantoin are compared for their effect on the faecal and periurethral flora in both domiciliary and hospitalized patients A small group treated with nalidixic acid is also included

PATIENTS

There were 37 boys and 4? girls are distribution is included in Table 4 Sixty nine had or had had a urinary infection 10 had respiratory infections The patients were divided into five groups with regard to drug treatment and hospital or domiciliary care sulphafurazole in hospital (24 patients 16 given 200 mg/kg/24 h 8 given 50 mg/kg/24 h) and at home (14 patients 200 mg/kg/24 h) nitrofurantoin 3 mg/kg/24 h in hospital (17 patients) and at home (19 patients) nalidixic acid 60 mg/kg/24 h in hospital (5 patients) Treatment was given for 10-12 days

Aided by the Swedish Medical Research Council (grant B71 19X 765 06B) and the Medical Faculty University of Goteborg

METHODS

Samples were taken on cotton wool swabs from the rectum and the periurethral area before during and on the last day of therapy and shaken in 1 ml of broth 0 1 ml of this was spread on each of the following four plates 1 lactose bromthymol blue agar (Drigalski) 2 sensitivity agar with 5 hors blood 3 same as 2 with 50 μg/ml of sulpha isodimidine 4 same as 2 with 50 μg/ml of nitrofurantoin The use of selective media made it possible to reveal th presence of resistant bacteria even in small numbers Standardized sensitivity discs were placed on plates 2 and 3 after drying Antibiotic and quantity in μg per disc were sulpha isodimidine (Su) 2 400 tetracycline (T) 50 chloramphenicol (C) 30 streptomycin (S) 50 ampicillin (A) 12 nitrofurantoin (Ni) 30 and nalidixic acid (Na) 30

All cultures were incubated aerobically and evaluated by K L In the faecal samples the quality and quantity of the growth was investigated and registered in such a way that the proportions between sensitive and resistant members of the aerobic Gram negative rod flora could be expressed as powers of ten The mere registration of absence or presence of resistant strains without regard to quantity gave essentially the same information The results of this simplified registration are shown in Fig 1

E coli O-grouping as well as testing for indole and ureas production was performed on at least two colonies with the appearance of *E coli* randomly chosen from non selective medium (11) Growth of

ZZ-individuals without liver disease three MZ-individuals and a control group have been examined clinically, histologically and immunohistochemically The clinical picture of the liver disease associated with Pi type ZZ in infancy was a neonatal cholestasis Alpha 1-antitrypsin was shown to be accumulated in the liver cells in an amount depending on the genotype—most in genotype ZZ less in genotype MZ, and independent of the degree of liver disease Evidence is presented that the alpha 1-antitrypsin deficiency in this syndrome is of the same genetic type as other ZZs In all cases the children had inherited one Pi^{Z} gene from each parent It is concluded that the pathogenesis of the alpha 1 antitrypsin deficiency is a mutation at the locus for the alpha-1-antitrypsin structural genes resulting in an abnormal protein which is released from the liver cells at a markedly reduced rate The tissue damage may arise by a nonspecific interference of the accumulated protein with vital intracellular processes or by excessive specific inhibition of important proteolytic enzymes in the hepatocytes The therapeutic implications of these hypotheses are discussed

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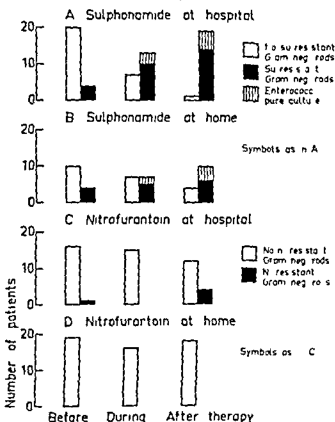


Fig 1 Sensitivity of the faecal flora in relation to therapy The nitrofurantoin resistant strains appeared in small amounts and belonged to genera other than *E coli*

enterococci was noted only if it clearly predominated over that of the enteric rods In the periurethral samples quality but not quantity was determined

RESULTS

Influence on faecal flora

Sulphonamide therapy Before sulphonamide therapy in hospital, *E coli* sensitive to sulphonamide dominated the aerobic flora (Fig 1) Resistant *E coli* were found in 4 patients in 3 of these however, the sensitive strains outnumbered the resistant ones At the end of therapy resistant Gram negative bacilli predominated in two thirds of the cases and in the other one-third there was a pure culture of sulphonamide resistant enterococci (Fig 1) The effect was the same whether a dose of 50 mg/kg (8 patients) or 200 mg/kg (16 patients) was used At dominant growth of sulphonamide sensitive *E coli* persisted in only 1 patient—none out of a thousand colonies was resistant Change of predominant *E coli* O group between the first and third faecal specimens, and sensitivity of the new sero groups is shown in Table 1 *Klebsiella* and/or *Proteus* were found in 2 patients before and in 6 after therapy (Table 2) No other enteric rods were found

In the domiciliary groups acquisition of resistant strains in patients with only sensitive ones in the first sample occurred in only 2 out of 10 as compared with 11 out of 17 in the hospital group The difference is close to significant ($p = 0.06$ χ exact calculation) The change of predominant *E coli* O group between first and third faecal specimen is shown in Table 1 No other enteric rods appeared (Table 2)

The high selective pressure of sulphafurazole is shown in Table 3 Persistence of the acquired resistant *E coli* was followed in 9 patients and was more than 2 months in 5 of them in 3 of these more than 5 months

Table 1 *Change of predominant* E coli *O group between 1st and 3rd faecal specimen*

Treatment group	Patients with change of O group/pat examined[a] No		χ^2	Sulphonamide resistance in new strains No	
Sulphafurazole in hospital	10/12	83	$0.2<p<0.3$	9/10	90
Sulphafurazole at home	5/8	63		3/5	60
Nitrofurantoin in hospital	5/15	33	$0.1<p<0.2$	0/5	—
Nitrofurantoin at home	2/18	11		0/2	—
Sulphafurazole total	15/20	75	p 0.001		
Nitrofurantoin total	7/33	21			

[a] Excluded from the analysis were patients with sulphonamide-resistant *E coli* in the pre in whom *E coli* disappeared during treatment or for whom the third specimen was missir

Table 2 *Gram negative rods other than* E coli *in faecal samples before and during or after therapy*

	Before therapy	During or after therapy
Hospitalized patients	4/46	13/46
Domiciliary patients	1/33	0/33

Table 3 *Selective pressure on faecal flora during 10–12 days of sulphafurazole therapy*

Pat no	*E coli* sero-group and resistance pattern	Resistant colonies/total number of colonies		
		Day 0	Days 3–5	Day 11
1	ON Su TCA	1/100 000	10/10	10/10
2	075 Su	3/5 000	8/10	—
3	011 Su	5/2 000	9/10	10/10

ON = *E coli* not groupable with the O-antisera 1 2, 3 4 6 7 8 9 18 25 75 nor with seven multivalent antisera to 56 other O groups

Nitrofurantoin therapy Before therapy the faecal growth was dominated by nitrofurantoin sensitive *E coli* in all hospitalized and domiciliary patients This was not changed by 10 days therapy (Fig 1) The O-antigen group of the predominant *E coli* strain was changed in 5 out of 15 hospitalized and in 2 out of 18 domiciliary patients investigated (Table 1) There is a significant difference between sulphonamide and nitrofurantoin with regard to influence on O group change (Table 1) A few colonies of nitrofurantoin resistant *Klebsiella/Enterobacter* were found in 1 patient before therapy After therapy 5 hospitalized patients had small amounts of resistant *Klebsiella/Enterobacter Pseudomonas Alcaligenes* and *Acinetobacter* (Fig 1)

Nalidixic acid therapy In 3 of 5 patients therapy was associated with appearance of a pure culture or dominant growth of Na resistant enterococci and/or alpha streptococci In the 2 others therapy was followed by dominant growth of nalidixic acid resistant *E coli* The changes in this small group thus appeared similar to those observed after sulphonamide

Periurethral flora

An analysis before therapy disclosed some differences related to age and sex (Table 4) Thus in boys below 1 year of age the mean number of different *E coli* strains per patient was higher than in boys over 3 years Gram negative rods other than *E coli* were found more frequently in boys than in girls (χ $0 02 < p < 0 025$)

Resistant strains were cultured more often after therapy than before (Table 5)

DISCUSSION

The effect of sulphonamide on the faecal flora of hospital patients in a previous study (11) was reproduced even when the dose was reduced from 200 mg/kg/24 h to 50 mg/kg/24 h The high selective pressure of sulphonamide shown in Table 3 corresponds with its high concentration in the gut during therapy (11)

Table 4 *Periurethral flora before therapy*

Age in years	Boys				Girls			
	<1	1–3	>3	Total	<1	1–3	>3	Total
No of patients	15	8	14	37	6	7[b]	29	42
Mean no of *E coli* strains per patient	1 6	0 25	0 07	—	1 0	0 5	0 8	—
Mean no of strains other than *E. coli* per patient	0 9	1 1	0 6	—	0 3	0 3	0.2	—

[a] Periurethral specimens obtained in 13
[b] Periurethral specimens obatined in 6
Klebsiella/Enterobacter (♂ 8 ♀ 2) *Proteus* (♂ 5 ♀ 3) *Pseudomonas* (♂ 1 ♀ 0) enterococci (♂ 8 ♀ 3) *Staphylococci* diphtheroids α-streptococci, *Candida* (♂ 7 ♀ 3)

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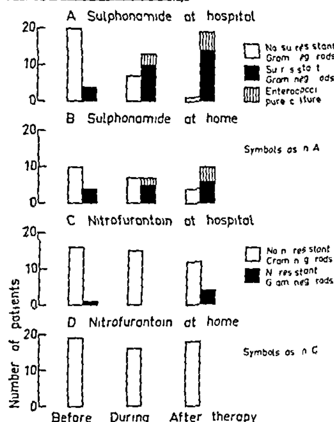


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Nitrofurantoin in hospital	5/15	33	$0.1 < p < 0.2$	0/5	—
Nitrofurantoin at home	2/18	11		0/2	—
Sulphafurazole total	15/20	75	$p < 0.001$		
Nitrofurantoin total	7/33	21			

[a] Excluded from the analysis were patients with sulphonamide resistant *E coli* in the pretreatment specimen patients in whom *E coli* disappeared during treatment or for whom the third specimen was missing

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Table 1 *Data on the children participating in the study*

Child	Sex	Age[a] (years)	Weight (kg)	Height (cm)	Surface[a b] (m)	Number of Weeks	Number of Days
D S	M	2 81	17.5	97 2	0 6695	2	13
J R	F	3 16	14 6	96 4	0 616	6	34
S S	F	4 11	16 2	107 2	0 684	1	7
G K	M	4 52	19 5	113 2	0 783	2	14
S C.	F	4 95	18 7	110 3	0 755	1	5
T G	F	5 06	18 7	113 2	0 769	1	7
J S	F	9 67	42.5	139 6	1 269	1	7
S M	F	11 28	32.6	146 3	1 172	3	18
A R	F	11 80	33 2	138 2	1 134	1	4
R R.	F	12 75	41.5	156 0	1 362	5	33
O M	M	13 42	33 4	137 7	1 134	1	7
Total 11						24	149

[a] Average for the period studied
[b] Body surface (cm^2) = weight ^{425} × height ^{725} × 71 84 (3)

with increasing age if calculated per body weight No such tendency is to be seen with the intakes calculated per body surface

For every single day the manganese content was related to the energy content The manganese contents of the diets averaged to 0 7–1 2 *mg/1 000* kcal There is no difference between the age groups

DISCUSSION

During the first three months of life breast fed infants receive 0 002 to 0 003 mg manganese per kg body weight and day Data on the manganese intakes of bottle fed infants are given as 0 006 or 0 05 mg/kg body weight and day (2 16 26) A strongly negative balance is reported for the age of 1 week (26)

Table 2 *Manganese intakes of children per day per calorie intake per weight and per body surface*

Child	Age (years)	Sex	Weeks	Manganese intakes: mg/day average	Range of intakes mg/day: Per day	Range of intakes mg/day: Average per week	mg/1000 kcal	mg/kg	mg/m²
D S	2 8	M	2	1 315	0 65–2 90	1 20–1 43	0 90	0 075	1 96
J R	3 ?	F	6	0 929	0 52–1 48	0 78–1 09	0 72	0 064	1 51
S S	4 1	F	1	1 732	1 05–3 35	—	*1 12*	*0 107*	2.53
G K.	4 5	M	2	1 687	0 90–2 55	1 32–2 05	0 95	0 086	2 15
S C	5 0	F	1	1 196	0 94–1 69	—	0 76	0 064	1 58
T G	5 1	F	1	1 548	1 16–1 99	—	1 10	0 083	2.01
J S	9 7	F	1	1 951	1 18–4 19	—	1 18	0 046	1.54
S M	11 3	F	3	2 013	0 80–6 33	1.30–3 01	1 02	0 062	1 72
A R	11 8	F	1	1 924	0 94–3 19	—	*0 89*	0 058	1 70
R R	12 8	F	5	1 981	0 91–9 40	1 51–3 01	0 98	0 048	1 45
O M	13 4	M	1	3 050	1 79–8 22	—	1 24	0 091	2 69
3–5 years									
Average				*1 40*	*0 52–3 35*	*0 78–2 05*	0 92	0 080	1 96
S D				0 31			0 16	0 016	0 38
9 13 years									
Average				2 18	0 80–9 40	1.30–3 05	1 06	0 061	1 82
S D				0 48			0 14	0 018	0 50

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MANGANESE IN THE DIET OF HEALTHY PRESCHOOL AND SCHOOL CHILDREN

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Since 1931, manganese has been regarded an essential trace element in animal nutrition Signs of manganese deficiency are similar in diferent species They include impaired growth of the skeleton and connective tissue degeneration of male and female reproductive organs, and ataxia of the newborn animal Carbohydrate and fat metabolism are influenced by manganese Pyruvate carboxylase was demonstrated to be a metallo enzyme containing manganese and arginase activity is influence by the same element (23)

Manganese deficiency has not been observed in man The manganese requirement is still under discussion data in the literature vary between 0 02–0 03 and 0 2–0 3 mg per kg body weight and day (1 4 8 9 12 21 28)

In the course of long term studies on the food intakes of healthy children fed ad libitum on a conventional diet (6) we analysed the manganese contents of daily food intakes of preschool and school children

MATERIAL AND METHODS

In the course of one year 149 daily food intakes of six children between 3 and 5 years of age and of five children between 10 and 13 years of age were analyzed The diet consisted of a varied mixed diet of the pattern usual in Westfalia The children received self selected amounts of the foods offered Occasional sweets and snacks were included in the diet Aliquots of the foods actually eaten were collected to daily food composites These were freeze dried ground and stored in airtight plastic bags for analysis A more detailed description of the sampling method is given in a preceding paper (20)

After mineralization of duplicate samples with HNO /HClO , manganese was determined by Atomic Absorption Spectrophotometry using standard methodology (3) From 20 duplicates the standard deviation of the method was calculated (11) as 0 013 mg/100 g dry matter The coefficient of variation was 2 5% The recovery was 100 3 on the average

Contamination with manganese during processing was prevented by keeping the sample in plastic containers and was compensated for by running blanks along with each batch of samples

RESULTS

Ages sex and selected anthropometric data of the children under study are given in Table 1 The children are the same studied previously (20)

Data on the manganese intakes are summarized in Table 2 The preschool children had about 1 4 mg manganese per day, the school children had about 2 2 mg per day There is an extreme day to-day variation of the manganese intakes The maximum intake is up to 10 times the minimum intake Even the averages over one week are still varying considerably

The average intakes of the children were between 0 05 and 0 1 mg manganese per kg body weight and day and 1 5 to 2 5 mg manganese per m body surface There is only a slight tendency towards decreasing intakes

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Table 1 *Data on the children participating in the study*

Child	Sex	Age (years)	Weight[a] (kg)	Height[a] (cm)	Surface[b] (m²)	Number of Weeks	Number of Days
D S	M	2 81	17 5	97 2	0 6695	2	13
J R.	F	3 16	14 6	96 4	0 616	6	34
S S	F	4 11	16 2	107.2	0 684	1	7
G K.	M	4 52	19 5	113 2	0 783	2	14
S C.	F	4 95	18 7	110 3	0 735	1	5
T G	F	5 06	18 7	113 2	0 769	1	7
J S	F	9 67	42.5	139 6	1.269	1	7
S M	F	11.28	32 6	146 3	1 172	3	18
A. R.	F	11 80	33 2	138 2	1 134	1	4
R. R.	F	12 75	41.5	156 0	1 362	5	33
O M	M	13 42	33 4	137 7	1 134	1	7
Total 11						24	149

[a] Average for the period studied
[b] Body surface (cm) = weight ⁵ × height ⁷² × 71 84 (3)

with increasing age if calculated per body weight No such tendency is to be seen with the intakes calculated per body surface

For every single day the manganese content was related to the energy content The manganese contents of the diets averaged to 0 7–1 2 mg/1 000 kcal There is no difference between the age groups

DISCUSSION

During the first three months of life breast fed infants receive 0 002 to 0 003 mg manganese per kg body weight and day Data on the manganese intakes of bottle fed infants are given as 0 006 or 0 05 mg/kg body weight and day (2 16 26) A strongly negative balance is reported for the age of 1 week (26)

Table 2 *Manganese intakes of children per day per calorie intake per weight and per body surface*

Child	Age (years)	Sex	Weeks	mg/day average	Manganese intakes: Range of intakes mg/day: Per day	Average per week	mg/1000 kcal	mg/kg	mg/m²
D S	2 8	M	2	1 315	0 65–2 90	1 20–1 43	0 90	0 075	1 96
J R	3 [illegible]	F	6	0 9[illegible]9	0 52–1 48	0 78–1 09	0 72	0 064	1 51
S S	4 1	F	1	1 732	1 05–3 35	—	1 12	0 107	2 53
G K	4 5	M	2	1 687	0 90–2 55	1 32–2 05	0 95	0 086	2 15
S C	5 0	F	1	1 196	0 94–1 69	—	0 76	0 064	1 58
T G	5 1	F	1	1 548	1 16–1 99	—	1 10	0 083	2 01
J S	9 7	F	1	1 951	1 18–4 19	—	1 18	0 046	1 54
S M	11 3	F	3	2 013	0 80–6 33	1 30–3 01	1 02	0 062	1 72
A R	11 8	F	1	1 924	0 94–3 19	—	0 89	0 058	1 70
R R.	12 8	F	5	1 981	0 91–9 40	1 51–3 01	0 98	0 048	1 45
O M	13 4	M	1	3 050	1 79–8 22	—	1 24	0 091	2 69
3–5 years									
Average				1 40	0 52–3 35	0 78–2.05	0 92	0 080	1 96
S D				0 31			0 16	0 016	0 38
9–13 years									
Average				2 18	0 80–9 40	1 30–3 05	1 06	0 061	1 82
[illegible]				0 48			0 14	0 018	0 50

Table 3 *Data on daily intakes of manganese*

Age	Sex	Weight[a] (kg)	Manganese intakes (mg/day)	(mg/kg body weight)	Remarks	Ref
1 week	M	(3 5)	(0 007)	0 002	Breastfed	(26)
1 month		4 0	0 011	0 003	Breastfed	(16)
1 month		4 0	0 024	0 006	Bottle fed	(16)
1–3 months		(5 0)	0 01	(0 002)	Breastfed	(2)
1–3 months		(5 0)	0 2	(0 04)	Bottle fed	(2)
3 months		5 7	0 014	0 0025	Breastfed	(16)
3 months		5 7	0 035	0 006	Bottle fed	(16)
1 year		(10)	0 8	(0 08)	Calculated	(2)
2 years			3 2	0 273	Analysed	(24)
3–5 years		*(16)*	*(3–5)*	*0 2–0 3*	*Proposed requirement*	*(9)*
3–5 years		17	1 4	0 08	This study	
6–10 years	F	28	2 1–4 8	0 08–0 17	Balance studies	(8)
7–9 years	F	28	2 3	0 08	Balance studies	(19)
7–9 years		(28)	1 7	(0 06)	Calculated	(2)
8–12 years			2 16	0 07	Balance studies	(14)
9 years			9 5	0 31	Analysed	(24)
10–13 years		37	2 2	0 06	This study	
16–19 years		(40)	6 52	0 16	Analysed	(27)
18–21 years	F	(50)	(2 5–4)	0 05–0 08	Balance studies	(18)
19–22 years	F	61	2 78	0 045	Balance studies	(15)
20–29 years	M		4 2–9 4	0 08–0 10	Balance studies	(13)
Adolescents	F			0 10–0 12	Balance studies	(22)
Adolescents	M			0 07–0 13	Balance studies	(22)
Students	F	(50)	< 0 24–1 53	(< 0 005–0 03)	Analysed	(25)
Adults		(70)	4 6	(0 07)	Derived requirement for balanced intake and excretion	(1)
Adults		(70)	3 7–5 8	(0 05–0 085)		(12)

[a] In brackets assumed weights and derived data

After the first year of life data in the literature on manganese intakes can be divided into two groups independent of age Some authors give rather high intakes of more than 0 1 mg manganese/kg body weight other give intakes of 0 05 to 0 08 mg/kg body weight per day Our results (Table 3) conform with the later group

The higher data were presented either some time ago or by authors from the USSR Differences in the dietary pattern may well play a major part for the explanation Germ and bran of cereals and pulses are rich in manganese Increased consumption of more refined cereal products leads to a low manganese intake Droste & Jekat (7) calculated the manganese intakes in households in West Germany These data show a decrease of the manganese intake of about 30% between 1950 and 1962

The manganese content of the adult human body is reported 20 mg (5) For a growing period of 15 years, we arrive at a daily needed *resorption of manganese for growth of only* 0 003 mg per day This figure is very close to the manganese intakes of breastfed infants during the first three months of life But it is only one thousandth of the daily intake reported for later periods of life

The manganese balance in man is characterized by the fact that most of the manganese is excreted in faeces Dependent on the manganese intakes the manganese content of faeces range from about 20 to 100% of the intakes (8 13 14 18 22 24) In most cases only 0 1 to 2% of the manganese intake is excreted in urine (10 14 22) The manganese content of sweat is about *0 06 mg/l (17)* Assuming a daily excretion of 350 ml sweat

by children (8) we calculate a daily manganese excretion through the skin of 0 02 mg/day

We plotted the manganese intakes given by Engel et al (8) for girls aged 6–10 years by North et al (18) for adult females and by Lang et al (13) for adult males against the corresponding retentions and found for all three groups that manganese intakes of 0 035–0 070 mg per kg body weight and day would result in balanced manganese intakes and excretions Based on the regression line calculated between manganese intakes and excretions Engel et al (8) arrived at a manganese requirement for girls aged 6–10 years of about 1 25 mg/day This corresponds to 0 045 mg/kg body weight and day

Compared to the requirement proposed by Engel et al (8) the intakes reported here may be regarded adequate

Some of the children are in the lower range One may wonder whether the tendency of decreasing manganese intakes may lead to manganese deficiency Along with Underwood (23) it is recommended to keep an eye on possible consequences of manganese undernutrition namely on the development of bones and cartilage during growth Possibly diabetes and increased fat deposits of the body should be checked on their relation to low manganese intakes

For children increased consumption of pulses and of cereal products of low extraction rate may be recommended

SUMMARY

In the course of one year the manganese contents of 149 daily food intakes of 11 children were analysed The average content of the diet was about 1 mg manganese per 1 000 kcal Children aged 3–5 years had an intake of 0 8–2 0 mg manganese per day children aged 10–13 years had 1 3–3 0 mg manganese per day Related to body weight the intakes were 0 05–0 1 mg/kg Related to body surface the intakes were 1 8–2 0 mg/m² The present trend of increased consumption of more refined food stuffs leads to reduced manganese intakes

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Key words Manganese intake preschool children school children manganese requirement

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PHYSICAL TRAINING MAXIMAL OXYGEN UPTAKE AND DIMENSIONS OF THE OXYGEN TRANSPORTING AND METABOLIZING ORGANS IN BOYS 11–13 YEARS OF AGE

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In industrialized societies many children live out their lives in urban areas Technical progress has facilitated their existence and reduced the amount of physical activity which was associated with a more primitive way of life The question then is to what extent has this development influenced their maximal aerobic power and the dimensions of their organs for oxygen transport and metabolism

The few longitudinal studies available on the effect of the training of children (5 8 15 19 22) have been focused on maximal aerobic power occasionally even including some of the dimensions of the oxygen transport organs The periphery i e the oxygen metabolizing muscles has never been included in any study Therefore the object of the present study is as follows

1 to consider the relationship between maximal oxygen uptake and the size of the oxygen transporting and metabolizing organs in a small sample of children from an urban area and

2 to examine the extent to which these variables and their inter relationship may change in conjunction with physical training

SUBJECTS

1? boys 11–13 years all students at the same elementary school volunteered for this study Although some of the boys belonged to a swimming club none performed any regular (hard) physical training Individual values for age weight and height are given in Table 1 and Figs 1 and 2 Medical examinations including ECG and routine blood and urine analyses were made before the study and all the boys were found to be healthy

METHODS AND PROCEDURE

Height (*H*) and weight (*W*) were carefully measured with double (sometimes triple) determinations at the beginning and at the end of the study The same measurement equipment was used Heart volume (HV) was determined in a prone position by means of biplane radiograms (14) Total haemoglobin (THb) was determined by the alveolar SO method (20) and double determinations were used Total lung capacity (TLC) was measured using the closed-circuit helium dilution method (10) Body potassium (K) was determined by whole body gamma radiation counting within a lead shield using a plastic scintillator in a fixed position over the subject sitting on a chair Th counting time was 40 min and the counts were analysed with a 100-channel pul e height analyser (7)

Maximal oxygen uptake was measured at least twice on an electrically braked bicycle *ergometer* (Elema) at a pedalling rate of 60 rpm Expired air was collected in Douglas bags and its volume measured in a balanced Tissot spirometer A modified Haldane technique was used for O and CO determinations Heart rate was obtained from ECG tracings and blood lactate was analysed using a colorimetric method (3) The lactate concentration value was only used as a check to ascertain if subjects had reached maximal aerobic power

The boys were examined from the middle of November to the end of December 1970 Physical training started in the middle of January 1971 and continued to the middle of May The same measure

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Table 2 *Individual values for training frequency and estimated intensity related to the change in maximal oxygen uptake per unit height squared*

Training intensity was graded on a scale of 1–5 with 5 as the highest intensity Numbers in parentheses indicate total number of training sessions held
Remark The April training camp is not included in the table

Subject	Number of training sessions Jan	Feb	March	April	May	Total	Estimated training intensity	Max $\dot{V}_{O_2}$ A/B corrected for growth (H^2)
	(5)	(12)	(7)	(7)	(3)	(34)		
R. F	5	12	6	4	3	30	2	0 9750
J H	5	12	7	6	1	31	3	1 0838
U H	5	11	7	7	2	32	2	1 0565
F L.	5	12	5	5	2	29	2	1 1619
T L.	5	10	7	6	0	28	4	1 0963
A M	3	12	7	6	1	29	5	1 4136
B M	5	11	5	7	1	29	5	1 1903
G P	5	11	5	7	1	29	3	1.24.0
P P	4	7	7	6	3	27	5	1 1652
Ri S	1	10	7	4	0	22	3	1 ?448
Ro S	5	12	6	5	2	30	3	1 0578
B A.	5	11	7	6	3	32	4	1 0492
				Mean		29 0	3 4	1 14428
				S D		2 7	1 2	0 11182
				S E.		0 8	0 7	0 03424

THEORETICAL CONSIDERATIONS

In the processing of measurement data our main problem concerned the fact that growth took place at the same time as the subjects were being trained Thus, there was a scale problem associated with the interpretation of our data and this fact governed our calculating procedure

All of the measured variables were expressed in units based on the fundamental units length mass and time The manner in which data of this type characterizing a dynamic system vary with the size of this *system is a problem* in the domain of classical physics The general solution to this problem was provided by Newton in his *Principia* (Book II Sect. 7 prop 32) See Whittaker (24) for a reformulation of Newton's *solution* (17) in modern mathematical notation

In applying physical laws to the size *problem* in work physiology the body should be regarded as a dynamic system in which the forces are proportional to the square of the linear dimension This statement is based on the theoretical view and the empirical fact that the force exerted by a *muscle* is proportional to its cross-sectional area It should also be remembered that the organism's tissue density is independent of size and mass scales, therefore equal the linear scales cubed Using *the* notation of dimensional analysis and applying the definition of force we can therefore write

$$\text{Force} = MLT^{-}$$

where L denotes the length dimension, M the mass dimension and T the time dimension Substituting the biological characteristics for force and mass we obtain

$$L^{2} = L\ L\ T^{-}$$

thus

$$L^{-} = T^{-2}$$

This means that the time scale in dynamic events of uniformly built moving organisms of different size is proportional to the length scale Corresponding analyses for special cases without referral to Newton's fundamental thesis have been made earlier (e g 1 6 12 13 16) The conclusions reached by these studies of special cases were the same as the general solution of the dimensional problem given here

Since both the mass scale and the time scale may be referred to the length scale in comparisons of individuals or groups of differing body size the relationship physically necessary to all data measured in units based on mass length and time may be derived If energy for example designated as $ML\,T^{-}$ is measured in subjects of differing size the measured values should be proportional to L flow per unit of time i e $L\,T^{-}$ should be proportional to L

If this approximation fails to accomodate empirical data then it should be concluded that the principle of similarity does not apply due perhaps to the interference of biological variation

In testing whether empirical data agree with the scale theorie or not a theoretical exponent of a relationship between variables shall be compared with an empirically found exponent

This means that the equation $y = ax$ shall be used for the different empirical data in the analysis The value for b best fitting the empirical data is then compared to the corresponding value according to the principle of similarity This is very simple in

Table 1 *Individual and mean values with S D and S E of variables before (B) and after (A) 4 months of physical training*

	Age (Years)		Height (*H*) (m)		Weight (*W*) (kg)		Potassium (K) (g)		Total haemoglobin (THb) (g)		Heart volume (HV) (ml)		Total lung capacity (TLC) (l)		Max O_2 uptake ($\dot{V}_{O_2\,max}$) (l/min)	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
R F	11 2	11 8	1 411	1 440	47 8	46 7	79 8	87 2	411	368	375	365	3 21	3 35	1 90	1 93
J H	11 0	11 6	1 375	1 409	35 0	33 1	74 4	81 5	302	304	310	385	2 71	3 08	1 52	1 73
U H	12 7	13 3	1 482	1 501	41 7	41 0	75 5	92 9	375	425	535	550	3 72	3 86	2 15	2 33
F L	11 3	11 9	1 562	1 601	44 0	45 7	83 3	93 4	381	444	500	535	3 71	3 97	1 77	16
T L	11 9	12 5	1 614	1 668	50 7	53 8	100 9	116 5	427	537	625	690	4 04	4 65	2 43	2 85
A M	12 9	13 5	1 608	1 664	47 9	52 3	87 3	105 4	457	512	505	535	3 66	4 29	1 73	2,6[illegible]
B M	11 5	12 1	1 614	1 655	57 3	55 0	91 4	102 2	407	482	600	705	4 38	4 92	1 81	2 79
Ro S	11 0	11 6	1 450	1 467	39 2	38 8	81 4	89 7	377	396	455	450	3 43	3 43	1 96	2 11
B Å	11 3	11 9	1 503	1 530	36 7	38 7	80 3	88 6	373	372	440	490	3 38	3 53	1 73	1 88
Mean (*n* = 9)	11 64	12 24	1 5132	1 5483	44 48	45 01	83 81	95 27	390 0	426 7	482 8	522 8	3 582	3 898	1 891	2 211
S D	0 71	0 71	0 0910	0 1014	7 18	7 66	8 32	10 84	43 44	75 2	101 0	118 6	0 481	0 622	0 267	0 359
S E	0 24	0 24	0 0303	0 0338	2 39	2 55	2 77	3 61	14 48	25 1	33 7	39 5	0 160	0 207	0 089	01 0
G P	11 1	11 7	1 438	1 459	47 9	49 8	—	—	381	412	550	590	3 54	3 26	1 69	2 16
P P	13 0	13 6	1 527	1 568	43 1	47 5	—	—	383	457	630	720	3 54	3 81	1 89	[illegible]8
Ri S	12 8	13 4	1 531	1 575	42 2	44 3	—	—	395	426	430	510	3 75	3 94	1 67	[illegible] 0
Mean (*n* = 12)	11 81	12 41	1 5096	1 5448	44 46	45 56	—	—	389 1	427 9	496 3	543 8	3 589	3 841	1 855	2 212
S D	0 81	0 81	0 0811	0 0910	6 26	6 71	—	—	37 22	64 9	99 3	117 1	0 414	0 562	0 242	0 307
S E	0 23	0 23	0 0234	0 0263	1 81	1 94	—	—	10 75	18 7	28 7	33 8	0 119	0 162	0 070	0 089

ments made before training were repeated within 3 weeks after the end of the training period and in the same order as before training

The subjects were trained three times a week for one hour per session There were 34 training sessions The boys participated well with mean attendance of 29 0 sessions (Table 2) In addition to the 34 training sessions a special training course in which all the boys participated was held in April at Vålådalen in the Swedish mountains A highly experienced physical education instructor with wide experience from similar studies was engaged Training procedures known to increase maximal aerobic power (2) were used Most of the training consisted of running at various speeds times and distances The duration of the rest pauses was varied In bad weather training was performed in a gymnasium using a vigorous program of gymnastics More intense training at least twice a day and mainly consisting of cross-country skiing was performed during the training camp stay

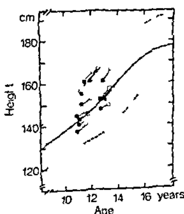


Fig 1 Individual values for height before (filled symbols) and after 4 months of physical training (unfilled symbols) Square symbols denote the three subjects for whom total potassium was not measured The solid line *indicates* mean height and broken lines ±2 S D of height for Swedish children according to Broman-Dahlberg-Lichtenstein (4)

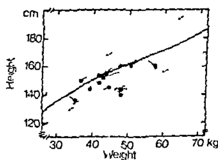


Fig 2 Individual values for weight plotted against height Symbols as in Fig 1 The solid line indicates mean weight for Swedish children according to Broman-Dahlberg-Lichtenstein Broken lines indicate ±2 S D and −3 S D of weight (4)

Table 7 suggested that no definite relation ship could be found between potassium in crease and testicular volume

The mean absolute 12 g increase in body ita sium corresponds to an absolute increase fat free tissue of about 5 kg i e about 4 ; if most of the increase was muscle Since ean weight increased by only 1/2 kg an erage of more than 3 kg of fat must have en lost during the training period which eans that more than 28 000 kcal (117 MJ) ad been metabolized from the body s fat ores It is questionable whether or not an crease in metabolism during training was e entire explanation for this loss

Maximal oxygen uptake increased by 12–4 more than could be ascribed to growth alculated per meter of height squared max mal oxygen uptake before training amounted o 0 83 l/min S D 0 121 and 0 92 l/min S D 098 after In spite of the obvious weakness of our material in the absence of a control group we attribute these changes to the effect of training Another study of training (8 9) observed that oxygen uptake per unit of height squared in nontraining 11 year old boys did not change over a period of 6 months

Table 5 *Correlation coefficients between vari ables before and after physical training inserted in the equation* $y=ax^b$

Parentheses indicate non significant *r*

x	*y* V_0 max	K	THb	HV	TLC	*W*
I *Before*						
K	(52)					
THb	(48)	69				
HV	67	74	67			
TLC	(58)	74	69	97		
W	(42)	75	76	68	79	
H	(35)	82	72	88	87	70
II *Afte*						
K	95					
THb	95	95				
HV	77	84	84			
TLC	83	90	92	94		
W	77	85	88	68	88	
H	81	90	9?	87	93	84

Table 6 *Regression coefficients (b) of the equa tion* $y=ax^b$ *with different variables before and after physical training*

Parentheses indicate non significant *r*

x	*y* V_0 max	K	THb	HV	TLC	*W*
I *Before*						
K	(75)					
THb	(57)	57				
HV	42	32	35			
TLC	(57)	51	59	1 56		
W	(36)	45	56	96	68	
H	(80)	1 30	1 39	3.25	2 00	1 86
II *After*						
K	1 38					
THb	84	58				
HV	54	41	67			
TLC	84	63	1 06	1 36		
W	71	54	92	90	80	
H	1 98	1 52	2 55	3 04	2 25	2 23

It should be noted that the increase in max imal oxygen uptake exceeded the increase in muscle mass raised to the appropriate power 2/3 derived from the principal of similarity This means that the efficiency of the muscles increased with respect to maximal power Since the maximal arterio-venous oxygen dif ference was of the same magnitude before and after training (11) an increase in the capacity of the muscles to utilize the oxygen transported to them by circulation may not be assumed Our conclusion is that changes during training which might explain the increase in maximal oxygen uptake should be sought in the circula tory system An increase in maximal cardiac output was also found in another study of the same group of subjects (11)

The dimensions of the circulatory and re spiratory organs did not change during the training period more than might be expected on the basis of growth alone This means that there must have been qualitative changes in the circulatory system

The results of regression analyses of the covariations of variables before and after training (Table 5) also call for comment The correlation coefficients between V_0 max and

Table 3 *Means of ratios between variables after (A) and before (B) physical training*

	A/B ($n=9$)	A/B ($n=12$)
H	1 0230 ± 0 00265	1 0232 ± 0 00224
H^2	1 0466 ± 0 00565	1 0456 ± 0 00457
H^3	1 0706 ± 0 00847	1 0672 ± 0 00707
W	1 0113 ± 0 01704	1 0245 ± 0 01491
K	1 1360 ± 0 01690	
THb	1 0899 ± 0 03738	1 0968 ± 0 02898
HV	1 0838 ± 0 02883	1 0963 ± 0 02336
TLC	1 0866 ± 0 02009	1 0689 ± 0 02025
$V_{O_2\,max}$	1 1733 ± 0 04930	1 1973 ± 0 03905

practice since the exponential equation in logarithmic form is graphically represented by a straight line When the logarithms of empirical data are subjected to regression analysis using the method of least squares the regression coefficient is the *b* of the exponential equation

RESULTS AND DISCUSSION

The individual values and the statistics for the measured variables are given in Table 1 Height and maximal oxygen uptake increased in all of the twelve subjects and body potassium increased in the 9 subjects in whom it was measured Total haemoglobin heart volume and total lung capacity increased in 10 of the subjects weight only increased in 7 subjects and decreased in 5

The ratio between individual values after and before the training period was calculated for each variable The squares and the cubes of height were also calculated The mean values for these parameters and their standard errors are given in Table 3 which shows a mean weight increase of 1–2%, increases of around 8% in height cubed, total haemoglobin heart volume and total lung capacity, a 14% increase in potassium and an increase in maximal oxygen uptake of 17–20%

A consistent part of the observed changes was apparently due to growth during the period of training In order to eliminate these changes from the discussion of the influence of training the ratios of the different variables after and before training were divided by the length scale raised to the appropriate power according to the principle of similarity The significance of the deviation from 1 0 in the values obtained in this manner was tested by Student s *t*-test The result was that no change could be demonstrated in total haemoglobin heart volume or lung capacity due to reasons other than growth alone Weight was down by about 5% potassium was 6% more and maximal oxygen uptake 12–14% more than expected (Table 4)

The 6% increase in body potassium points to an increase in muscle mass since 2/3 of body potassium is located in the muscle cells Whether or not any increase in muscle mass was due to the training is another question Since muscularity may be a secondary sexual characteristic and testosterone has been shown to have some influence on muscle growth (21 22) the change in potassium content in our subjects was related to their testicular volume However the results of this test shown in

Table 4 *Means of ratios between variables after (A) and before (B) physical training corrected for body size*

	A/B ($n=9$)			A/B ($n=12$)		
Variables	Mean	t	Significance	Mean	t	Significance
W/H^3	0 9446	4 072	**	0 9602	3 063	**
K/H^3	1 0614	3 658	**	—	—	—
THb/H^3	1 0176	0 548	N S	1 0274	1 110	N S
HV/H^3	1 0121	0 485	N S	1 0275	1 287	N S
TLC/H^3	1 0046	0 360	N S	0 9938	0 450	N S
$V_{O_2\,max}/H^2$	1 1174	2 747	*	1 1424	4 116	**

group took place if accordance with the assumption of similarity is to be taken as a criterion of normality The changes in some covariations during training are graphically illustrated in Figs 3 and 4

This leads to the question of what is normal in children of this age their status before or after training? Even if this may be a question of the definition of normality we should here like to stress that the living conditions of the subjects with respect to physical activity were abnormal before training in the sense that the sedentary way of life in urban areas should not be regarded as normal for children of this age The change in thier body composition and physical performance capacity reflected their normalization as a result of physical training

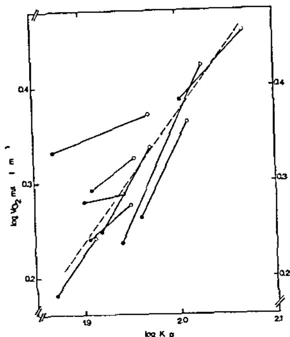


Fig 4 Logarithms of individual values for max V_{O_2} and total potassium before (filled symbols) and after 4 months of physical training (unfilled symbols) Broken line represents regression equation after training (V_{O_2}max = *0 00409* $K^{1\ 89}$)

SUMMARY

Twelve boys 11–13 years living in an urban area were subjected to physical training for 4 months Height weight testicular volume total haemoglobin heart volume total lung capacity total potassium and maximal oxygen uptake were measured before and after the training period

During the period of observation mean height increased by 3 5 cm This introduced a scale problem in the interpretation of the data Therefore due attention had to be paid in the analysis to Newton s principle of similarity in dynamic systems The following results were obtained

Total haemoglobin heart volume and total lung capacity failed to increase more than could be explained by growth alone

Body weight only increased sligthly and significantly less than might be expected from growth

Body potassium increased more than could be explained by growth indicating a change in body composition with increased muscle mass and decreased fat

Maximal oxygen uptake increased by 12–14 % more than could be explained by growth Height squared was found to be a better parameter of V_{O} max than weight V_{O} max/height squared was 0 83 l min^{-1} m^{-} before training and 0 92 l min^{-1} m^{-} after

The agreement of measurements with the principle of similarity was tested by regression analysis of the logarithms of experimental data and found to be better after training than before

Since the way of life during the training period may be considered as being more normal for boys of this age than their sedentary lives before training the data found before training are not acceptable as normal values

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Table 7 *Increase in K (%) after physical training of boys grouped according to testicular volume (TV cm³) (18) after training period*

	TV<4 8	4 8<TV<9 0	TV>9
R F	9 3		
J H	9 6		
U H	23 0		
F L		12 1	
T L			15 5
A M			20 0
B M			11 8
Ro S		10 2	
B A	10 3		

body size variables were low before training and only significant for heart volume This circumstance changed after the training period when these correlation coefficients all became significant and very high (0 95) for potassium and total haemoglobin It is also remarkable that the correlation of all the variables with height was better than correlation with weight

Since the logarithms of the measured values were used for the regression analysis, study of the regression coefficient (Table 6) in comparison with the anticipated theoretical value obtained from the assumption of similarity is of special interest We used the values after training since the correlation coefficient was higher after training than before

Let us first consider the covariation between height and the other variables The value for b with V_{O} max as y is 1 98 which is very close to the theoretical value 2 With potassium as y however the exponent is only 1 52 as opposed to the anticipated 3 This means that the assumption of similarity does not apply to muscular development The muscles of taller boys are relatively thinner than those of shorter boys This is in definite contrast to the case of the circulatory variables The exponents for total haemoglobin and heart volume were 2 55 and 3 04 respectively which were not far from anticipated values

If we again turn to potassium we find that the exponent in covariation with maximal uptake is 1 38 which is twice the expected 2/3 This fact showed that our test subjects were not scale models of one another with respect to utilized muscle mass at maximal aerobic power At maximal work the taller subjects used a greater percentage of their available muscle mass for oxygen consumption than the shorter subjects This is not consistent with the theory that muscle mass might be the limiting factor for maximal aerobic power On the other hand the figures do not contradict the theory that the dimensions of the circulatory organs limit maximal aerobic power after training

In comparing the regression coefficients before and after training we found that the relationship among the variables generally shifted towards better agreement with the approximation of similarity The exponent of the relationship weight (y) and height (x) increased from 1 86 to 2 23, the theoretical value should be 3 The corresponding value for total haemoglobin and height increased from 1 39 to 2 55 etc It appears as if a normalization of the

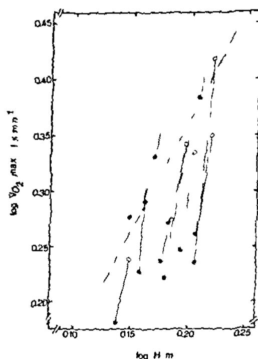


Fig 3 Logarithms of individual values for height against maximal oxygen uptake Filled symbols indicate values before training unfilled symbols after training Broken line represents theoretical covariation between log height and log $\dot{V}_{O_2}$max (Regression coefficient ~2)

roup took place if accordance with the assumption of similarity is to be taken as a criterion of normality The changes in some observations during training are graphically illustrated in Figs 3 and 4

This leads to the question of what is normal in children of this age their status before or after training? Even if this may be a question of the definition of normality we would here like to stress that the living conditions of the subjects with respect to physical activity were abnormal before training in the sense that the sedentary way of life in urban areas should not be regarded as normal for children of this age The change in thier body composition and physical performance capacity reflected their normalization as a result of physical training

SUMMARY

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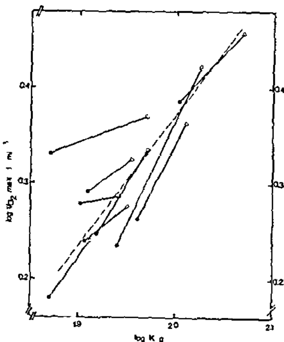


Fig 4 Logarithms of individual values for max V_{O_2} and total potassium before (filled symbols) and after 4 months of physical training (unfilled symbols) Broken line represents regression equation after training (V_{O_2}max = 0 00409 $K^{1 \cdot}$)

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FIBRIN DEGRADATION PRODUCTS AND PLASMINOGEN IN NEWBORN INFANTS WITH RESPIRATORY DISTURBANCES AND POSTNATAL ASPHYXIA

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Recent advances have widened our knowledge of the significance of fibrin/fibrinogen degradation products (FDP) in various diseases in adults such as cancer renal disorders and thrombosis (18)

In newborns interest in FDP has been centred mainly on conditions in which disseminated intravascular coagulation (DIC) is apt to occur e g in haemorrhagic diathesis septicaemia and idiopathic respiratory distress syndrome (1 7 8 9 15 16 17 23)

In a previous study at this laboratory (12) it was shown that sera from healthy newborns do not contain any appreciable amounts of FDP

It was thought worthwhile to continue these studies on sick newborns and to relate the findings to events during pregnancy delivery and the first minutes of life This paper concerns determinations of FDP and plasminogen within the first 48 hours of life in preterm and term infants with and without certain common neonatal disorders

CLINICAL MATERIAL

The material consisted of 154 infants with gestational age between 25–42 weeks They were examined at the Departments of Paediatrics in Malmo and Umeå between April 1968 and July 1971

The distribution of the infants according to gestational ...

The infants were referred to one of the following two groups according to their clinical condition

I *The asymptomatic group* consisted of 64 infants who had Apgar scores (3) of 7–10 at 10 min and who were afterwards apparently healthy

II *The symptomatic group* consisted of 90 infants with Apgar scores of <7 at 10 min and/or who later developed symptoms compatible with the following diagnoses

1) Idiopathic respiratory distress syndrome (IRDS) 34 infants 18 of whom died The diagnosis was based on clinical findings meeting the criteria of Hutchison et al (20) and/or a patho-anatomical finding of typical hyaline membranes 3 of these infants had concomitant intracranial haemorrhages and are not included in group 4

2) Respiratory distress syndrome (RDS) 17 infants all surviving IRDS was clinically probable but the diagnosis was not verified by X ray and therefore did not satisfy all the criteria for IRDS

3) Unclassified respiratory symptoms (URS) 35 infants 8 of whom died a heterogeneous group not meeting the criteria for groups 1 and 2 consisting of infants with, e g attacks of cyanosis apnoea aspiration syndrome

4) Intracranial haemorrhages (ICH) 4 infants 2 of whom died In the 2 cases in which the infants survived the neonatal period the diagnosis was based on positive findings at a lumbar or subdural puncture In the other 2 cases the diagnosis was verified at autopsy

None of the 154 infants had neonatal infections hypoglycaemia isoimmunizations or congenital malformations

28 infants died in the neonatal period Two of them had pulmonary haemorrhages one together with intracranial haemorrhages the other together with URS

The infants were also grouped with respect to the course of pregnancy and/or delivery The asymptomatic and the symptomatic group were thus each

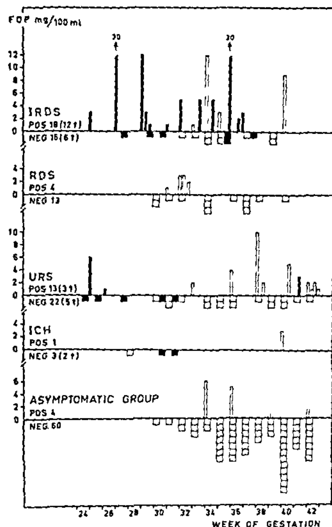


Fig 1 Distribution of the material according to gestational age of the infants and diagnosis Every case is included the FDP pos ones by the columns above the lines (amounts of FDP mg/100 ml) the FDP neg ones below the lines by squares Open symbols surviving infants filled symbols infants who died For abbreviations IRDS RDS URS ICH see text Preterm less than 39 weeks of gestation term 39–42 weeks where week 40 = days 274–280

divided into four subgroup (Tables 1 and 2) The complications of pregnancy were both internal and obstetrical (Table 2) Complications of delivery consisted of malpresentations and other difficult deliveries e g protracted labour cord around the neck instrumental delivery and caesarean section A low Apgar score *per se* was not classified as a complication of delivery

Blood sampling

All blood samples were obtained within the first 48 hours of life from an indwelling plastic catheter in one of the umbilical vessels When possible the samples were obtained at the same time as those for blood gas analyses A sample was obtained on only one occasion from 92 infants but on 2 to 6 from the remaining 62 In 19 cases sampling was continued beyond the age of 48 hours

LABORATORY PROCEDURES

FDP and plasminogen were determined in serum from blood collected in a tube with an inhibitor of fibrinolysis E aminocaproic acid (EACA) Serum was frozen immediately after centrifugation of the blood and stored at −20 C until analysed The following methods were used

Fibrin/fibrinogen degradation products (FDP) immunochemical method (27) The normal range of variation of FDP was determined in a previous investigation of healthy infants born at term (1?) In the present study FDP values of ≥1 mg/100 ml after 2 hours of life were regarded as abnormal FDP demonstrated only within the first 2 hours of life were not accepted as being of any clinical significance Cases filling and not filling this criterion are here referred to as "FDP pos and FDP neg respectively

Plasminogen immunochemical method (12 14) The means and S D given in Figs 2 and 3 for different gestational ages were obtained from a previous study of plasminogen (10) They were as follows <33 weeks 24 0±8 4 33–35 weeks 30 3±9 8 36 38 weeks 34 7±11 1° 39–42 weeks 43 4±16 0°

pH and blood gas determinations arterial blood samples were drawn into a heparinized glass syringe and analysed between 30 minutes and 1 hour after collection The various assays used were those described by Koch & Wendel (24)

All determinations were made as part of the routine care of the infant The data obtained were compared with the normal values given by Koch & Wendel (24) for the first week of life pH Po Pco and standard bicarbonate were measured but in the following presentation only pH and Po are taken into account Only values below −3 S D were accepted as clearly abnormal and indicating acidosis and hypoxia respectively

Table 1 *Distribution of FDP positive and negative cases in relation to complications of pregnancy and/or delivery*

N = Normal C = Complication

		Asymptomatic group		Symptomatic group	
Pregnancy	Delivery	pos	neg	pos	neg
N	N	1	25	10	12
C	N	—	12	4	15
N	C	1	11	11	11
C	C	2	12	11	16
		4	60	36	54

Statistical methods

The significance of differences between the various groups of FDP positive and FDP negative cases were tested with the ordinary χ^2 test. Wilcoxon's rank sum test was used to test the significance of differences between the amounts of FDP in the groups of surviving and dead infants.

RESULTS

I. FDP determinations

The total material is shown in Fig. 1, where all infants are presented in relation to gestational age and diagnosis. The amounts of FDP in the positive cases are also given. When more than one sample was obtained the highest value was used.

The distribution of FDP positive and negative cases in relation to complications during pregnancy and/or delivery is given in Tables 1 and 2. Such complications occurred in 38 cases in the asymptomatic group and in 68 in the symptomatic group. No relation could be demonstrated between FDP findings in the infants and the presence or absence of complications during pregnancy and/or delivery.

FDP in the asymptomatic and in the symptomatic group (Table 1). 4 of 64 infants in the asymptomatic group and 36 of 90 in the symptomatic group were FDP positive. This difference is significant ($p < 0.001$). Data on the 4 FDP positive cases in the asymptomatic group regarding gestational age and complications during pregnancy/delivery are summarised in Fig. 1 and Tables 1 and 2. The amounts of FDP varied between 2 and 6 mg/100 ml.

In the symptomatic group the range of FDP was 1–12 mg/100 ml among the surviving infants and 1–30 mg/100 ml among the infants who died. The difference between the two latter groups was not significant. However, the largest amounts of FDP (30 mg/100 ml) were found in two infants who died.

FDP findings in the various groups of diagnoses in infants are presented in detail in Fig. 1 and summarised in Tables 2 and 3. Infants with IRDS who died (12 pos/6 neg) were FDP positive in a significantly larger proportion ($p < 0.01$) than the rest of the symtomatic group (24 pos/48 neg) and those in the total surviving group (21 pos/41 neg, $p < 0.02$). Such differences were not found for any of the other groups of diagnoses, nor did the distribution of FDP positive and negative cases between the total groups of surviving infants and infants who died show any significant differences.

FDP findings versus Apgar scores at 1 and 10 minutes are given in Table 4. The distribution of FDP positive and negative cases in the group of infants with Apgar scores of 0–3 at 1 and 10 min (8 pos/0 neg) differed significantly ($p < 0.001$) from that in the group with scores of 7–10 at both 1 and 10 min (10 pos/25 neg). Taken together, the group of infants with 0–3 points at 1 min but 4–6 or 7–10 points at 10 min (18 pos/4 neg) were FDP positive in a significantly larger proportion ($p < 0.001$) than that which received 4–6 or 7–10 points at both 1 and 10 min (18 pos/50 neg). Those infants with Apgar scores of 7–10 at 10 min in the symptomatic group (17 pos/44 neg) were also compared with the asymptomatic group (4 pos/60 neg) and were shown to contain significantly more FDP positive cases ($p < 0.005$).

FDP findings versus arterial pH and blood gas determinations (Table 5). Such assays were performed in 73 of the 90 infants in the symptomatic group. In 13 infants the assays were done only once, whereas in the remaining 60 they were done two or more times during the first 48 hours of life. The infants were divided into three groups with respect to pH and blood gas findings (Table 5). The "extreme" group contained infants where the limits for pH and P_O were arbitrarily chosen as ≤ 7.10 and ≤ 30 mmHg respectively. No significant differences in the distribution of FDP positive and negative cases could be demonstrated between the three groups. There were relatively more FDP positive infants within the group with "extreme" acidosis but that group was too small to warrant statistical evaluation.

Table 2 *Number of FDP positive cases in the various groups of complications of pregnancy and delivery*

The number of FDP positive cases/number of infants is given for each group Note that the number of infants in each row in the table exceeds the total number of infants since complications of pregnancy and delivery occurred in 14 cases in the asymptomatic group and in 27 cases in the symptomatic group

			Complications of								
			Pregnancy				Obstetrical				
			Internal								
	No complications	No of infants	Toxaemia	Hepatosis	Diabetes	Others	Placenta praevia	Abruptio placentae	Ab imm + bleedings	Premat. rupt membr	Others
Asymptomatic group	1/26	38	1/8	0/2	0/2	0/3	—	1/2	0/7	0/2	—
Symptomatic group	10/22	68	2/6	0/2	2/2	—	1/3	2/5	3/14	4/8	1/6

2 *Serial plasminogen determinations*

Plasminogen was assayed on 2 or more occasions in 74 infants (26 in the asymptomatic group and 48 in the symptomatic group) The results were divided into three categories (*a*) the asymptomatic group (Fig 2) (*b*) the FDP positive cases and (*c*) the FDP negative cases of the symptomatic group (Fig 3) The two latter parts contained each 6 deaths The results were also grouped with respect to gestational age it having been shown in a previous study (10) that plasminogen increases significantly with gestational age

No differences could be demonstrated between the three categories Infants who died showed the same plasminogen levels as the surviving infants of corresponding gestational age Plasminogen levels in single cases varied more than ±2 S D in 6 infants (one in the asymptomatic group 4 FDP pos and one FDP neg infant in the symptomatic group)

Table 3 *FDP positive and negative cases of the symptomatic group in relation to further course and the various diagnoses*

Outcome	Surviving		Dead		No of infants
FDP	pos	neg.	pos	neg	
IRDS	6	10	12	6	34
RDS	4	13	—	—	17
URS	10	17	3	5	35
ICH	1	1	—	2	4
Total	21	41	15	13	90

Table 4 *Distribution of FDP positive and negative cases in relation to Apgar scores at 1 and 10 minutes in the symptomatic group*

Apgar score 1 min / 10 min→ ↓	FDP pos /neg			
	0–3	4–6	7–10	Total
0–3	8/0	9/2	1/2	18/4
4–6	0/0	1/4	6/17	7/21
7–10	0/1	1/3	10/25	11/29
Total	8/1	11/9	17/44	36/54

DISCUSSION

Fibrin/fibrinogen degradation products (FDP) may appear in serum because of intravascular or extravascular breakdown of fibrinogen or fibrin The fibrinolytic activity responsible for this breakdown may be produced in the general circulation (primary fibrinolysis) or locally in the walls of vessels where fibrin and thrombi are deposited (secondary fibrinolysis) In the few investigations in newborn infants where FDP have been studied in serum collected with an inhibitor of fibrinolysis both these possibilities have been suggested as the cause of FDP Thus Ludwig (25) felt that asphyxiated

Delivery			
Instrumental	Difficult'' deliveries	Caesarean section	
0 3	2/15	1/8	
4 4	8/23	10/2?	

newborns have an increased fibrinolytic activity a hypothesis for which he produced both experimental and clinical evidence On the other hand according to Hathaway et al (17) and Chessells & Wigglesworth (7 8 9) disseminated intravascular coagulation (DIC) is the most probable cause of FDP

In our investigation we measured FDP within the first 48 hours of life We found a significantly larger number of FDP positive cases in a symptomatic group of infants than in an asymptomatic group the proportions being about 40 and 6% respectively The frequency of FDP positive cases reported by Altstatt et al (2) among infants with IRDS and other neonatal disorders was much lower than in our symptomatic group Comparison between our studies is however difficult because of different methods used

No relation was found between the presence of FDP in the infants and the complications during pregnancy and/or delivery This finding suggests that FDP do not pass the placenta barrier since pregnant women with various complications often have FDP in the serum (5 19) Support for this concept has recently been presented in an experimental study on pregnant sheep and their foetuses (4) It is thus probable that FDP found in the infant during the first 48 hours of life really originate from some fibrin/fibrinogen degradation process in the infant itself

In this respect it was considered of interest to relate FDP findings to the Apgar score which is a good measure of the infant s clinical condition FDP were significantly more common in infants with an Apgar score of 0–3 at 1 min than in those with a higher score at that time At 10 min the frequency of FDP positive cases decreased with increasing Apgar score It was however still significantly higher than in the asymptomatic group

A low Apgar score indicates the existence of some pathologic condition which may perhaps be reflected by the presence of FDP Our findings would then be in agreement with the experience from this laboratory where comprehensive investigations of FDP in adults (18) with Nilehn s immunochemical method (27) have shown that FDP are never demonstrable in health and that when present they always denote disease

We found no correlation between acidosis/hypoxia and the presence of FDP As pre

Table 5 *Distribution of FDP positive and negative cases in relation to arterial pH and P_o determinations*

≥ -3 S D no acidosis/hypoxia < -3 S D acidosis/hypoxia present extreme group pH $\leq 7\ 10$ $Po_2 < 30$ mmHg

FDP	≥ -3 S D				< -3 S D (except extreme group)				Extreme group			
	pH		Po		pH		Po		pH		Po_2	
	pos	neg.	pos	neg.	pos	neg	pos	neg.	pos	neg.	pos	neg
No of infants	[illegible]	13	19	33	14	23	4	5	7	6	3	4

Table 2 *Number of FDP positive cases in the various groups of complications of pregnancy and delivery*

The number of FDP positive cases/number of infants is given for each group Note that the number of infants in each row in the table exceeds the total number of infants since complications of pregnancy and delivery occurred in 14 cases in the asymptomatic group and in 27 cases in the symptomatic group

			Complications of								
			Pregnancy								
			Internal				Obstetrical				
	No com plica tions	No of infants	Tox aemia	Hepa tosis	Dia betes	Others	Placenta praevia	Abruptio placentae	Ab imm +bleed ings	Premat rupt membr	Others
Asympto matic group	1/26	38	1/8	0/2	0/2	0/3	—	1/2	0/7	0/2	—
Sympto matic group	10/22	68	2/6	0/2	2/2	—	1/3	2/5	3/14	4/8	1/6

2 *Serial plasminogen determinations*

Plasminogen was assayed on 2 or more occasions in 74 infants (26 in the asymptomatic group and 48 in the symptomatic group) The results were divided into three categories (*a*) the asymptomatic group (Fig 2) (*b*) the FDP positive cases, and (*c*) the FDP-negative cases of the symptomatic group (Fig 3) The two latter parts contained each 6 deaths The results were also grouped with respect to gestational age, it having been shown in a previous study (10) that plasminogen increases significantly with gestational age

No differences could be demonstrated between the three categories Infants who died showed the same plasminogen levels as the surviving infants of corresponding gestational age Plasminogen levels in single cases varied more than ±2 S D in 6 infants (one in the asymptomatic group 4 FDP pos and one FDP neg infant in the symptomatic group)

Table 3 *FDP positive and negative cases of the symptomatic group in relation to further course and the various diagnoses*

Outcome	Surviving		Dead		No of infants
FDP	pos	neg	pos	neg	
IRDS	6	10	12	6	34
RDS	4	13	—	—	17
URS	10	17	3	5	35
ICH	1	1	—	2	4
Total	21	41	15	13	90

Table 4 *Distribution of FDP positive and negative cases in relation to Apgar scores at 1 and 10 minutes in the symptomatic group*

Apgar score 1 min / 10 min→ ↓	FDP pos /neg			
	0–3	4–6	7–10	Total
0–3	8/0	9/2	1/2	18/4
4–6	0/0	1/4	6/17	7/21
7–10	0/1	1/3	10/25	11/29
Total	8/1	11/9	17/44	36/54

DISCUSSION

Fibrin/fibrinogen degradation products (FDP) may appear in serum because of intravascular or extravascular breakdown of fibrinogen or fibrin The fibrinolytic activity responsible for this breakdown may be produced in the general circulation ('primary' fibrinolysis) or locally in the walls of vessels where fibrin and thrombi are deposited ('secondary' fibrinolysis) In the few investigations in newborn infants where FDP have been studied in serum collected with an inhibitor of fibrinolysis both these possibilities have been suggested as the cause of FDP Thus Ludwig (25) felt that asphyxiated

No significant differences were found between the different groups of diagnoses or between the infants who survived and those who died. Neither did the amounts of FDP differ significantly between the groups. But the infants who had IRDS and who died in the neonatal period were FDP positive significantly more often than the rest of the symptomatic group and the total group of survivors. Our results are at variance with those of Hathaway et al. (17). These authors found that only a small proportion of the patients who probably had DIC and who died showed FDP, whereas most infants without evidence of DIC were found to have FDP. They felt that one reason for this difference might be a depletion of fibrinolytic activity in infants with severe IRDS, which was found by Markarian et al. (26) and recently also by Karitzky et al. (22). In our material, however, FDP were often found shortly before death.

The plasminogen levels in the asymptomatic and symptomatic groups and in the infants who survived and those who died fell within the same ranges when related to gestational age. A postulated deficiency of fibrinolysis in sick newborns can therefore, in our opinion, not be ascribed to abnormally low plasminogen levels. Our results are at variance with those of Karitzky et al. (21), who found the highest plasminogen levels in healthy prematures and the lowest ones in those who died from IRDS and intracranial haemorrhage. Two explanations may be offered for the difference in our results. First, we collected the blood for plasminogen determinations in the presence of E-aminocaproic acid, which prevents plasminogen activation *in vitro*. Second, we correlated the plasminogen levels with gestational age, whereas Karitzky et al. correlated them with birth weight, which is a less reliable measure of maturity (13).

In conclusion, judging from our study, the frequency of FDP in a given series of sick newborns are unquestionably more common than in "healthy" newborns even if both groups have experienced the same complications of pregnancy and delivery. We also found a higher frequency of FDP in infants with low Apgar scores and in those who died from IRDS. But FDP can obviously appear also in infants with other respiratory disorders and even in those who have mild symptoms and survive. Their presence may indicate DIC, but bouts of primary fibrinolysis or local tissue damage may be other possibilities. The finding of FDP in infants with IRDS may be a sign of a bad prognosis. The demonstration of FDP must always be regarded as being of pathological significance. This concept agrees well with the occurrence of FDP found at our laboratory in the investigation of adults. Plasminogen was found in the same amounts in the infants, irrespective of symptoms or further course, in the different gestational age groups.

SUMMARY

Fibrin/fibrinogen degradation products (FDP) and plasminogen were studied in 154 infants with gestational ages between 25 and 42 weeks. The material was divided into two parts: an asymptomatic group of 64 infants who were apparently healthy during the neonatal period and a symptomatic group of 90 infants who developed IRDS, unspecific respiratory symptoms and intracranial haemorrhages. Mothers of infants of both the asymptomatic and the symptomatic groups had had various complications during pregnancy and/or delivery. Determinations of the arterial pH and blood gases were made in 73 of the 90 infants in the symptomatic group. FDP, assayed from 2 to 48 hours of life, were significantly more common in the symptomatic than in the asymptomatic group. No correlation was found between the presence of FDP in the infants and the complications during pregnancy and/or delivery, suggesting that FDP originate from some fibrin/fibrinogen degradation process in the infant. FDP were significantly more common in infants with low Apgar scores. No significant correlation could be found between FDP and acidosis/hypoxia. The

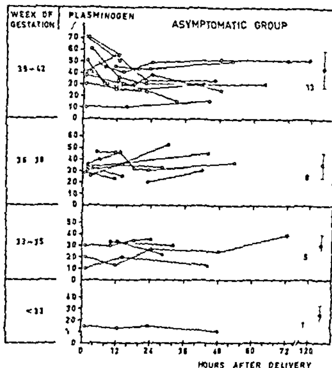


Fig 2 Serial plasminogen determinations in 26 infants of the asymptomatic group classified according to gestational age Mean and ±S D (see text) of each gestational age group (10) is given *n* = number of infants in the present study Filled symbols FDP pos aliquot of sample used for determining plasminogen

viously pointed out (11), a low Apgar score at 1 min does not reflect oxygenation itself (3) and does not permit any conclusions about a causal relationship between hypoxia and the presence of FDP as proposed by Chadd et al (6) Our results make it questionable whether moderate acidosis and hypoxia are involved in the causation of fibrinolysis and FDP later in the neonatal period It must be remembered that blood gas analyses merely give an instantaneous picture of the infant s condition and are not sufficient to describe the course of the disease, in which other factors may be responsible for the appearance of FDP In single cases, however the association between a high fibrinolytic activity and FDP with "extreme" acidosis and hypoxia seemed striking but may perhaps merely be different expressions of a common disorder The two highest FDP values (30 mg/100 ml) were found in infants who died of IRDS with extreme acidosis It is conceivable that acidosis/hypoxia induces a high fibrinolytic activity only when it is so pronounced as to produce tissue damage

SYMPTOMATIC GROUP FDP NEGATIVE CASES — PLASMINOGEN % — WEEK OF GESTATION — PLASMINOGEN % — SYMPTOMATIC GROUP FDP POSITIVE CASES

39–42 · 36–38 · 33–35 · <33

IRDS · RDS · URS

HOURS AFTER DELIVERY

Fig 3 Serial plasminogen determinations in 48 infants of the symptomatic group classified according to gestational age and diagnosis (IRDS RDS URS) 23 FDP pos and 25 FDP neg cases Mean and ±S D as in Fig 2 *n* = number of infants in the present study Filled symbols FDP pos aliquot of sample used for determining plasminogen

newborn infant during the first week of life *Biol Neonat 12* 136 1968
25 Ludwig, H Mikrozirkulationsstorungen und Diapedeseblutungen im fetalen Gehirn bei Hypoxie *Bibl Gynaec* vol 46 Karger Basel New York 1968
26 Markarian M Githens J H Jackson J J Bannon A E Lindley A Rosenblut, E. Martorell R & Lubchenco L. O Fibrinolytic activity in premature infants Relationship of the enzyme system to the respiratory distress syndrome *Am J Dis Child 113* 312 1967
27 Nilehn, J E Separation and estimation of split products" of fibrinogen and fibrin in human serum *Thromb Diath Haemorrh 18* 3 1967

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highest proportion of FDP positive cases occurred in infants who died from IRDS Otherwise no significant differences were found between the groups of diagnoses or between the infants who survived and those who died Neither did the amounts of FDP differ significantly between the groups

FDP in sick newborns may appear in both severe and mild cases Their presence may indicate DIC, but episodes of a more marked fibrinolytic component or local tissue damage are other possibilities The appearance of FDP in infants with IRDS may be a bad prognostic sign and must generally speaking be regarded as being of pathological significance

The plasminogen levels in the asymptomatic and symptomatic groups and in the infants who survived and those who died fell within the same ranges when related to gestational age A postulated deficiency of fibrinolysis in sick newborns can therefore not be ascribed to lower plasminogen levels than in healthy newborns

ACKNOWLEDGEMENTS

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RENAL RESPONSE TO AN ORAL SODIUM LOAD IN NEWBORN FULL TERM INFANTS

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In the newborn infant renal function is reported to be restricted with regard to filtration rate (16, 22), concentrating (10) and acidifying capacity (11). Knowledge about the renal control of sodium excretion in the infant kidney is, however, limited.

The present study is an attempt to assay the ability of the newborn full term infant to excrete sodium following a standardized oral load of sodium chloride. As a reference the renal effect of a quantitatively corresponding load to older children has been used (2, 5).

MATERIAL

Thirty one healthy infants have been studied. The age distribution is shown in Fig. 1. The gestational age ranged between 38–42 weeks according to case history, weight and length and the result of neurological evaluation of the maturity. Twenty seven of the infants had a normal delivery, 2 were delivered with vacuum extraction and 2 with caesarean section. None of the children had Apgar score below 7 at one minute after birth, all of them had 10 after five minutes. All infants had been fed with breast milk or formula.[1] The studies were carried out in the nursery ward. The body temperature did not change and the babies were disturbed as little as possible during the course of the study. They showed no signs of discomfort. No case of diarrhoea was encountered. One baby who vomited during the study was excluded. Informed maternal consent was given in all cases studied.

EXPERIMENTAL

In order to ensure a good and relatively constant diuresis all studies were carried out during standardized fluid expansion. The infants were fed by stomach tube. During the entire course of study they were given a standardized composition of diluted formula (Allomin diluted 1:3). The diluted formula was calculated so no baby should receive less than 60 kcal/kg body weight and day during the study. The first hour of the study the infants were given the diluted formula in an amount corresponding to 2 % of the body weight, followed every 30 min by an amount corresponding to 0.5 % of the body weight. Urine was obtained by spontaneous voidings at approximately hourly intervals. A high and relatively stable diuresis was achieved after about 90 min.

The infants were divided into the following three groups.

Group I (20 babies). After the collection of at least one urine sample an oral sodium load consisting of 95 mEq Na^+ per 1.73 m^2 body surface (0.12 g sodium chloride per kg body weight) was given. The sodium chloride was dissolved in the diluted formula, yielding a 1 % saline solution. This modified formula was administered during a 30 min period.

Group II (5 babies). Same as group I, except that 190 mEq Na^+ per 1.73 m^2 was given. The sodium chloride was dissolved as described above, yielding a 2 % saline solution.

Group III (6 babies). Same as group I, except that no extra sodium chloride was added to the formula.

After the administration of the sodium chloride load urine was collected for 5–8 hours.

In 5 babies from Group I the glomerular filtration rate was determined by analyzing the disappearance rate of a single intravenous dose of inulin. Following the injection, capillary blood was obtained every 5–10 min for 80 min. Inulin (Laevasan Gesellschaft) in a 10 % solution was given in an amount of 0.75 ml per kg body weight. The values of GFR were calculated from the formula given by Sapirstein (18).

This work has been supported by grants from Semper Research Foundation and Karolinska institutet.

[1] Allomin (Semper), sodium content 6.5 mEq/l, or Milkotal (Findus), sodium content 8.7 mEq/l.

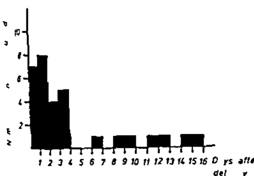


Fig 1 Age distribution of the infants studied

$GFR = I \times \gamma_A \times \gamma_B / A \times \gamma_B + B\ \gamma_A$

(*I* the amount of inulin injected)

A typical disappearance curve in a representative infant is shown in Fig 2

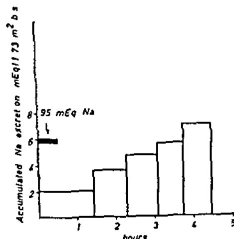


Fig 3 Accumulated Na⁺ excretion in a 6-days old infant after the administration of a 1% saline solution

ANALYTICAL METHODS

he sodium concentration in serum and urine was ialyzed by a flame photometer (Eppendorf) Inulin i blood was determined according to Heyrovsky (12) smolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer Hema xcnt in capillary blood samples was estimated in lass capillaries which were centrifuged at 10 000 :PM for 5 min

RESULTS

Urine sodium excretion

Fig 3 demonstrates the hourly accumulated sodium excretion in a representative study of an infant from Group I The hourly sodium excretion seemed to be fairly constant from 1 to 5 hours after the sodium load and no peak excretion was observed It therefore seemed justified to calculate the average hourly sodium excretion from the total excretion between 1 and 5 hours after the load had been given

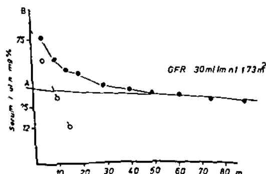


Fig 2 Plasma disappearance curve of inulin following an intravenous injection of a 10% inulin solution Filled circles represent actual plasma values, unfilled circles constructed line representing actual disappearance rate from blood compartment. The scale is in log line

The mean values of the hourly sodium excretion following a load with water or saline solution (1 and 2) are given in Table 1 As a reference the table also includes the average hourly sodium excretion following a corresponding salt load in children aged 8 to 14 years (5) The urinary sodium excretion was

Table 1 *Sodium excretion mEq per hour per 1 73 m body surface following an oral load of water or saline chloride solution*

The average hourly urine Na excretion ± one S D in infants during high fluid intake with and without an oral salt load The urinary Na⁺ excretion following an oral salt load in older children is given as a reference

Load	High fluid intake only	High fluid intake + 95 mEq Na⁺	High fluid intake + 190 mEq Na⁺
Infants			
1–15 days	0 53±0 09	1 51±0 28	1 11±0 52
Children			
8 14 years	6 0±2 1	16 0±1 8	25 7±3 5

higher after a salt load than when only water had been given However, since the sodium excretion did not differ significantly after a load of one or two per cent saline solution the ability to excrete sodium does not seem to be dose dependent The natriuretic response to a salt load in infants was significantly lower than it was in older children following the corresponding load The average hourly urinary excretion of sodium which was found in infants 1–15 days after birth is seen in Fig 4 No increase of the ability to excrete an oral sodium load was noticed As seen in Fig 5 there is a significant inverse correlation between the sodium excretion and the hematocrit ($p < 0.001$ correlation coefficient $= -0.778$)

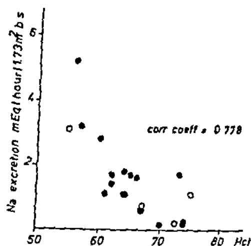


Fig 5 The relationship between the hematocrit and the average hourly Na^+ excretion following a 1% (filled circles) or a 2% (unfilled circles) saline load The correlation coefficient −0.778, was statistically significant

Glomerular filtration rate

Glomerular filtration rate (GFR) was determined during the course of the oral sodium chloride load in 5 infants As is seen from Table 2 the values of GFR ranged between 20–34 ml per minute per 1 73 m² No correlation between sodium excretion and glomerular filtration rate was observed

Urine dilution

Table 2 also includes the average values for diuresis urine osmolality and sodium clearance during the course of the study

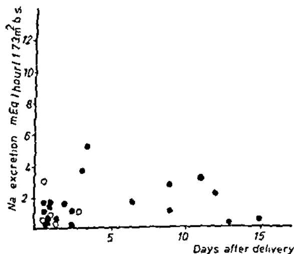


Fig 4 The average hourly Na^+ excretion following the administration of a 1% (filled circles) or a 2 (unfilled circles) saline load to infants aged 1–15 days

From the osmolality determination of the hourly sampled urine osmolar clearance (C_{osm}) and free water clearance (C_{H_2O}) can be calculated using the formula

$C_{osm} = (U_{osm} \times V)/B_{osm} \quad C_{H_2O} = V - C_{osm}$

where V_{osm} and B_{osm} stand for urine

and blood osmolality respectively and V stands for the diuresis Likewise the sodium clearance can be calculated using the sodium concentration in the hourly sampled urine The sum of free water clearance and sodium clearance is generally assumed to be an index of sodium delivered to the distal tubule Free water clearance as such is generally used as an index of distal tubular sodium reabsorption (20 3) If free water clearance is plotted against the sum of free water clearance and sodium clearance a characteristic relationship is generally found suggesting that distal tubular sodium reabsorption can be increased as a function of distal tubular sodium availability

Fig 6 demonstrates this relationship in the 5 infants included in Table 2 as compared with that found in healthy young adults It is apparent from the figure that free water clearance in infants is on the upper limit or higher than the free water clearance found in healthy young adults at each level of the sum of free water clearance and sodium clearance This

Table 2 *Glomerular filtration rate urinary sodium excretion and urine osmolality in 5 infants following a 1 % saline load*

Sex	Age (days)	GFR (ml/min/ 1 73 m²)	Na excretion (mEq/hour/ 1 73 m²)	Average diuresis[a] (ml/min)	Average urine osmolality[a]	Average Na[a] clearance (ml/min)
Male	3	31	2 8	0 75	61	0 056
Femal	9	20	1 1	0.24	70	0 016
Male	11	24	3 2	1 07	43	0 054
Male	13	34	0 3	0 30	55	0 005
Male	15	20	0 5	0 52	47	0 003

Average value from 2–6 urine collection periods

suggests a more complete reabsorption of sodium in the distal tubule of the infant than in the distal tubule of the adult

DISCUSSION

The extremely low sodium concentration in human milk 7 mEq/l may suggest a reduced ability of the infant kidney *to excrete sodium* The finding of an extremely low sodium elimination rate following an acute saline load was therefore not surprising The finding is also in agreement with the results from a study in piglets on the effect of an oral sodium chloride load (15) The piglets developed edema if they were fed with 0 5 % saline solution instead of corresponding amounts of sow's milk It is well established that the glomerular filtration rate of the newborn infant is low (25–35 ml per minute per 1 73 m body surface) (16 22) The infants in the present study had a filtration rate between 20 and 34 ml per minute per 1 73 m body surface It has recently been shown in this laboratory that the ability to excrete an oral sodium load is restricted in older children with low filtration rate secondary to renal disease (2) The restriction of the sodium elimination rate is in fact directly related to the glomerular filtration rate Thus the sodium elimination rate in those children with glomerular filtration rate between 20–35 ml per minute per 1 73 m² body surface ranges between 6–10 mEq per hour per 1 73 m² body surface In the infants however the sodium elimination rate was 0 1 to 3 mEq per hour per 1 73 m body surface Thus in the infant kidney the reduction of the sodium elimination rate is out of proportion to the reduction of the glomerular filtration rate and must be attributed to a specific inability of the tubules to reject sodium adaptively

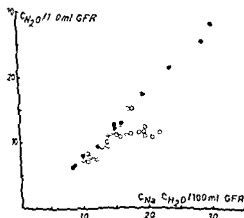


Fig 6 The relationship between the sum of sodium and free water clearance ("distal tubular Na+ delivery") and free water clearance ("distal tubular Na reabsorption") Filled circles represent values obtained in 5 infants unfilled circles values obtained in 14 healthy young adults (3)

Since extracellular volume expansion has a natriuretic effect which is independent *of the* glomerular filtration rate (9) the control of *tubular* sodium reabsorption has been subjected to extensive *investigations During* the past years the following factors have all been attributed some importance for the control of tubular sodium reabsorption

higher after a salt load than when only water had been given However, since the sodium excretion did not differ significantly after a load of one or two per cent saline solution the ability to excrete sodium does not seem to be dose dependent The natriuretic response to a salt load in infants was significantly lower than it was in older children following the corresponding load The average hourly urinary excretion of sodium, which was found in infants 1–15 days after birth is seen in Fig 4 No increase of the ability to excrete an oral sodium load was noticed As seen in Fig 5 there is a significant inverse correlation between the sodium excretion and the hematocrit ($p < 0.001$, correlation coefficient $= -0.778$)

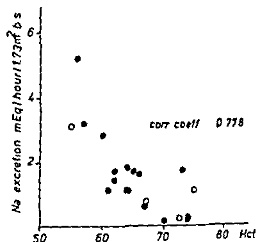


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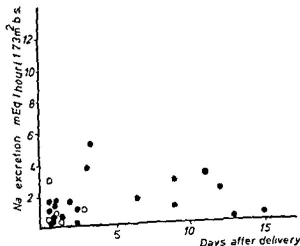


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of 20–35 ml per minute per 1 73 m body surface area at a blood sodium level of about 140 mEq per liter the maximal sodium excretion can be calculated to be about 12 mEq per kg body weight per 24 hours It may also be pointed out that water is always passively reabsorbed with sodium at least in the proximal tubule Thus enhanced sodium reabsorption is not necessarily associated with increased serum sodium concentration but may result in an almost iso-osmotic fluid retention of varying degree most seriously manifested by the development of edema Another clinical aspect so far only open to speculation is the possibility that a moderately positive sodium balance during early infancy might result in future arterial hypertension (8)

SUMMARY

Urinary elimination rate of a standardized oral sodium load has been studied in 31 full term healthy infants aged 1–15 days

The elimination rate of sodium was found to be low in newborn infants as compared to what has been observed in older children The reduction was out of proportion to the restriction of the glomerular filtration rate

Various possible explanations for the reduced ability of the immature renal tubule to reject sodium adaptively such as the high hematocrit and a different distribution of the intrarenal blood flow have been discussed

The apparent implication of the finding is a low tolerance to sodium in the newborn baby The maximal tolerated daily dose of sodium has been calculated to be about 12 mEq per kg body weight

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1 Mineral corticoid steroid hormone This factor can hardly be responsible for the sodium retention observed in infants since aldosteron secretion rate has been shown to be low during the first week of life (21)

2 Physical forces It is well established that intrarenal physical forces such as peritubular capillary and interstitial hydrostatic and oncotic pressure influence tubular sodium reabsorption (4, 6, 14) Polyglobulia is a condition in which physical forces have been shown to enhance tubular sodium reabsorption (7, 19) The mechanisms behind this effect is thought to be as follows high hematocrit results in increased filtration fraction increased filtration fraction results in increased peritubular capillary oncotic pressure a situation known to enhance tubular sodium reabsorption The hematocrit of newborn infants is high In the present study a significant correlation was found between hematocrit and tubular sodium reabsorption suggesting that physical forces are at least partially responsible for the enhancement of tubular sodium reabsorption in newborns It should be pointed out however that the effect of the hematocrit on tubular sodium reabsorption in infants is probably less pronounced than in the experimental conditions with polyglobulia since the plasma oncotic pressure as such is low in infants In addition the enhancement of distal tubular sodium reabsorption suggested by the analyses of free water clearance cannot be attributed to the effect of physical forces since so far micropuncture studies have failed to reveal any important effect of this factor on the distal tubule (1)

3 Intrarenal blood flow distribution It has been claimed that intrarenal blood flow distribution to a certain extent will influence the overall glomerular tubular balance for sodium (17) It is generally agreed that the juxtamedullary nephrons have a higher capacity to reabsorb sodium than the cortical nephrons If a relatively high proportion of the renal blood flow would perfuse the juxtamedullary nephrons a relatively large proportion of the filtered load of sodium would be reabsorbed That this mechanism might be responsible for the enhancement of the overall sodium reabsorption in small infants is suggested by a recent study in newborn puppies (13) Using ^{133}Xenon wash out—as well as microsphere technique—it was clearly demonstrated that during the first weeks of life cortical blood flow is low in relationship to total blood flow and will increase markedly after an age of 10–12 weeks

4 A natriuretic hormone Presently there are no conclusive evidence of the existence of such a hormone (23)

To sum up The results from the present study do not reveal the exact mechanism of the relative inability of kidney tubules of newborn infants to reject the reabsorption of sodium adaptively It is likely that changes in physical forces brought about by the high hematocrit are at least partially responsible for the enhancement of tubular sodium reabsorption A relative increase in the proportion of renal blood flow perfusing the juxtamedullary nephrons might however also contribute to th increased fractional sodium reabsorption during the first weeks of life

The present study has clearly shown that the infant kidney as compared to the mature kidney tends to retain sodium This suggests a reduced tolerance of the newborn baby to salt administration The most acutely riskful clinical situation is undoubtedly presented by the seriously acidotic baby to whom repeated doses of sodium bicarbonate is administered Previous observations have demonstrated that older children can excrete at most 20% of the filtered load in situations of acute salt overloading (unpublished observations in this laboratory) During the first weeks after birth the elimination rate of an acute sodium load was approximately 25% of that found in older children with corresponding glomerular filtration rates Thus the newborn infant should in the situation of a high salt load only be able to excrete about 5% of the filtered load of sodium Assuming a glomerular filtration rate

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TECHNICAL ASPECTS AND RESULTS OF REGULAR HEMODIALYSIS IN CHILDREN

M BROYER C LOIRAT and C KLEINKNECHT

From the Clinique des Maladies du Rein et du Metabolisme chez l'Enfant Paris France

Treatment of terminal uremia by long term hemodialysis and transplantation is now universally performed in adults Application of these procedures to children began more recently Technical problems questionable long term results and fear of major psychological involvement explain this delay However though some children have been treated in adult centres paediatric hemodialysis units have now sprung up (1 3 4)

From January 1969 to March 1971 30 children and adolescents with terminal stage kidney disease were referred to our department Five of them were excluded from the hemodialysis program two because of mental retardation one because of extrophy of urinary bladder one because of oxalosis one after refusal of treatment by parents All other children without any prior social or psychological selection were submitted to chronic hemodialysis in view of subsequent kidney transplantation

CASE MATERIAL

Twenty five children and adolescents with terminal uremia (creatinine clearance less than 5 ml/min/1 73 m²) aged 20 months to 20 years have been treated by regular hemodialysis Primary disease age sex weight and duration of dialysis are indicated on Table 1 Each child is dialysed two or three times weekly for 10 to 20 hours (20 to 30 hours per week)

METHODS

The technique of hemodialysis in children is not very different from that in adults

Cannula

Under local or more frequently general anesthesia cannulation of vessels was always possible with the standard Silastic Teflon arterio-venous Quinton Scribner cannula (7) Generally the smallest tip models (cannula no 16-18) were used For children over 20 kg the cannula was inserted into the radial artery and a superficial forearm vein of the non-dominant hand In 2 cases the cannula was inserted into the internal tibial artery and the external saphenous vein after iterative cannulation of the forearm vessels In 3 children weighing 8 (cases 12 and 20) and 15 kg (case 19) the cannula was inserted into the brachial artery and a cephalic or a deep vein of the arm In 11 children over 16 kg the venous dilatation secondary to the creation of a subcutaneous arterio-venous fistula between the radial artery and a superficial vein of the forearm allows easy venopuncture for each dialysis Adult venous fistula cannulation sets (Travenol 18/10 mm) are used in children over 30 kg In four children weighing 16 to 30 kg smaller needles ("Butterfly" Abbott 16/10 mm) are used

Delivery system

The dialysate solution is delivered at a rate of 700 ml/min by machines which mix softened tap water with a commercially prepared concentrate so that the final formula is sodium 132 mEq/l potassium 1 or 2 mEq/l chloride 100 5 mEq/l acetate 38 mEq/l magnesium 1 5 mEq/l calcium 3 mEq/l This delivery system contains a pump which allows negative pressure from 0 to −250 mmHg adjustment being done according to the desired water substraction

Dialysers

From January 1969 to July 1970 three types of dialyser were used the standard two-layer kiil dialyser a pediatric modified two-layer kiil dialyser shorter than the previous one and the eight layer disposable Rhone Poulenc dialyser Only the latter has been used since July 1970 As can be seen on Table 2 one layer of either normal or pediatric kiil dialyser implies an extra corporeal blood volume which would be too important for small children On the opposite with

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Table 1 *Patient data*

Patient	Age (y)	Age (mo)	Sex	Weight (kg)	Initial disease	Duration of hemodialysis (months)	Current status
1	17	4	M	47	Membrano proliferative GN[a]	13	Rejection 1 year after live transplantation regular hemodialysis in adult centre
2	20	1	M	45	Primary NS[b]	25	6 months after cadaver transplantation
3	9	9	F	25	Rapidly progressive proliferative GN	1½	25 months after cadaver transplantation
4	13	4	F	34	Nephronophthisis	26	Regular hemodialysis
5	12	7	M	28	Proliferative GN	24	Regular hemodialysis
6	13	10	M	43	Hypoplasia with dysplasia	1 (23)[c]	Regular hemodialysis
7	9	5	F	22	Hemolytic-uremic syndrome	23	Regular hemodialysis
8	14	10	F	41	Segmental hypoplasia	6 (29)	Regular hemodialysis
9	17	10	M	35	Primary NS	8 (29)	Regular hemodialysis
10	9	8	F	21 5	Segmental hypoplasia	23	Regular hemodialysis
11	14	4	M	32	Unknown	22	Regular hemodialysis
12	1	8	F	8	Hemolytic-uremic syndrome	21	Regular hemodialysis
13	13	1	M	37	Schönlein Henoch purpura	19	Regular hemodialysis
14	13	1	F	22	Urinary tract malformation	12	Died
15	15	4	M	41	Unknown	1 (15)	Regular hemodialysis
16	11	9	F	16	Hypoplasia	13	Regular hemodialysis
17	14	9	M	35	Focal glomerular hyalinosis	12	Regular hemodialysis
18	11	9	F	30	Rapidly progressive proliferative GN	11	Regular hemodialysis
19	3	11	M	15	Chronic interstitial nephritis	11	Regular hemodialysis
20	2	5	M	8	Hypoplasia	6	Regular hemodialysis
21	15	11	M	40	Diffuse renal arteriolar thrombosis of unknown origin	4 (6)	Regular hemodialysis
22	14	1	F	26	Nephronophthisis	4	Regular hemodialysis
23	12	8	M	27	Urinary tract malformation	2	Regular hemodialysis
24	12	2	F	23	Nephronophthisis	½	Regular hemodialysis
25	3	11	F	16 5	Oxalosis	½	Regular hemodialysis
					Total	289 5	

[a] GN = Glomerulonephritis
[b] NS = Nephrotic syndrome
[c] Figures in parentheses represent total duration of regular hemodialysis carried on in other centre

the Rhone Poulenc dialyser one can adjust the extra corporeal blood volume to the patient s size This is particularly important in young children if preserved blood is not used for priming Using a single layer of this dialyser and connecting tubing of reduced length and diameter one can cut extracorporeal blood volume down to less than 100 ml Each extra layer adds 35 ml to the extracorporeal blood pool Thus one may choose the number of layers to be used in a given patient according to his weight the optimum ratio being in the range of 10 to 15 ml per kilogram

Diet and treatment

A diet is prescribed in every case with 1-3 g/kg of proteins according to age Fifty to sixty percent of this amount must be of animal origin Dietary sodium (0 3-2 mEq/kg) is adjusted to natriuresis and to arterial blood pressure and dietary potassium is limited to 1-2 mEq/kg Average theoretical caloric intake is 70 calories/kg/day, with a range of 34 to 130 calories/kg day Diet is maintained during dialysis but the child is allowed to eat what he wishes within the first 4 hours Polyvitamin mixture is regularly added in twice the physiological doses as well as calcium supplementation (total calcium intake 450-1 300 mg/day)

Exchange resin (sodium polystyrene sulfonate) is prescribed so that predialysis potassium level does not reach 6 mEq/l Aluminium hydroxide is given so that predialysis phosphorus levels is kept between 50 and 70 mg/l Vitamin D_2 supplementation is variable though a 3 mEq l dialysate calcium concentration has been shown to allow a positive calcium balance during dialysis (11) it seems that vitamin D resistance observed in uremia still has to be compensated by vitamin D_2 supplementation In practice Vitamin D doses were 4 to 12 000 units/day in children without radiological bone lesions and with normal alkaline phosphatases In the other cases this dose must be raised to 16-20 000 units day

RESULTS AND DISCUSSION

nnula

nnula problems have been numerous (Fig Clotting was the most frequent complica n (41 episodes) generally due to angulation cannula or to progressive sclerosis of the ssel as shown by shuntograms It led 33 nes to reinsertion of the cannula on 15 oc sions reinsertion was possible in the same ssel at a more proximal site in 18 cases an her vessel had to be cannulated Clotting was equally frequent in the three smallest chil ren with a brachial shunt in case 12 can ula survival was fairly satisfactory on the op- osite iterative clotting (7 episodes within 6 onths) led to abandon hemodialysis and to esume peritoneal dialysis in case 20 Clotting as also very frequent in case 19 No acute rophic problems have appeared in children vith brachial shunts Accurate information oncerning member growth is not available at resent

Skin infection and necrosis led to reinsertion of cannula on 8 occasions 4 times in another vessel of the same arm and 4 times of the other arm Two septicemias were due to cannula problems In one case (no 11) staphylococcal septicemia due to skin infection led to abla tion of shunt and reinsertion in the other arm In another case (no 18) pyocyanic septicemia occurred after successful declotting manipula tions During the next 2 months carbenecillin treatment could not be stopped without im mediate septicemic recurrence Recovery was rapidly obtained after ablation of shunt and substitution of peritoneal dialysis for hemo dialysis for 1 month An arteriovenous fistula was subsequently created So for 211 patient months experience average cannula life was 4 9 months for arterial cannula (26 reinsertions or abandons) and 4 months for venous can nula (36 reinsertions or abandons)

These problems concerning shunts led to the creation of a subcutaneous arteriovenous fis tula in all children except nos 19 and 20 One month after surgery venous dilatation was sa

Table 2 *Extracorporeal blood volume of dialysers*

Type of dialyser	Extracorporeal blood volume (ml)
Kiil	
Tubing	140
One layer	250
Two layers	500
Pediatric kiil	
Tubing	140
One layer	110
Two layers	220
Rhône–Poulenc dialyser	
Normal Tubing	90
Pediatric tubing	60
One layer	35
to	to
Eight layers	280

tisfactory even in small children Passage from shunt to arterio-venous fistula is appreciated as a liberation by all children in spite of a per sistent apprehension of needle insertion in a few cases particularly in small children However the long term psychological incidence of iterative venipuncture is still unknown

One must add that regular controls of car diothoracic ratio and of cardiac output have shown no modifications which could be at tributed to the shunts and/or fistulas but the follow up period is probably too short

Blood flow rate

Flow rates of 100 to 200 ml/min were obtained with Scribner shunts without using a pump A flow rate under 100 ml/min suggests that blood flow through the cannula is not satis factory because of angulation or because of vessel sclerosis Both may lead to clotting if not corrected A blood pump was occasionally useful during dialysis in these cases to prevent clotting in the dialyser

Flow rates of 100 to 200 ml/min were also obtained with fistulas for which a pump is al ways necessary

Dialysance of artificial kidneys

Urea dialysance performances obtained with the three types of dialyser were compared (Fig 2) It appears that for an equal extracorporeal

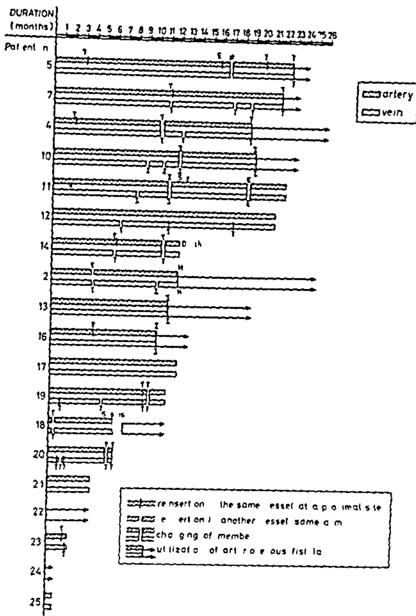


Fig 1 Cannula survival *T* = Thrombosis *I* = Skin infection and necrosis *E* = Progressive extrusion *H* = Peri cannula bleeding ■ = Post traumatic hematoma and necrosis T⁺ = Thrombosis during transplantation

blood volume, best results are obtained with the Rhone Poulenc dialyser and second with pediatric kiil

Biochemical results

Mean results concerning urea, creatinine, uric acid and potassium for 1969 and 1970 are reported in Table 3 Average Blood Urea Nitrogen was 100 mg before dialysis and 31 mg/100 ml after dialysis during 1969 and 93 mg/100 ml before dialysis, 21 mg/100 ml after dialysis during 1970

Clinical results

Clinical response to dialysis was good in most cases 1 to 4 weeks after the first dialysis all children but 4 (cases 7 12 14 21) regained a good physical activity and could be sent home They now attend school normally Symptoms of pericarditis, present in 4 cases disappeared within 2 to 4 weeks of dialysis Good rehabilitation was retarded by severe osteodystrophia in 1 case (no 14 see later) and by cachexia bound to malignant hypertension in 3 cases (nos 7 12, 21)

No major complications were due to dialysis itself Still discomfort related to disequilibrium syndrome with headaches and vomiting was often noted during last hours of dialysis The weight gain between dialysis was generally 1 to 2 kg in children over 25 kg 0 5 to 1 kg in smaller children Weight remained

Table 3 *Biochemical data*
BUN = Blood urea nitrogen mg/100 ml K = Blood potassium in mEq/l U ic ac d in mg/l p e D post D = p e and po t di ly i

	1969						1970						
		BUN		K				BUN		K			
Patients no	Duration (months)	pre D	post D	pre D	post D	Uric acid pre D	Duration (months)	pre D	post D	pre D	post D	Uric ac d pre D	creatinine pre D
2	12	89	32	5	3 8	88	12	108	37	5 2	3 7	88	139
4	11	95	32	4 9	3 8	75	12	105	21	4 9	3 5	72	149
5	9	84	33	5	3 9	73	12	86	24	4 5	3 6	71	121
7	8	96	29	5 9	4 1	82	12	82	22	5 6	3 7	83	92
10	8	1 0	35	5 1	3 7	71	12	117	24	4 9	3 4	82	145
11	7	176	43	5 7	4	99	12	107	23	4 8	3 4	87	124
12	6	107	22	5 8	3 5	90	12	81	13	5 4	3 4	83	77
13	4	94	31	4 3	3 8	92	12	107	28	4 9	3 7	91	123
14	2	93	29	5 3	3 8	58	10	81	23	4 3	3 2	73	90
16							10	83	12	5 3	3 3	97	96
17							9	90	28	4 4	3 4	99	137
18							8	75	17	3 9	3 2	78	92
19							8	115	17	4 3	3 2	83	94
20							3	97	12	5 4	3 3	86	54
21							3	93	27	5 2	3 6	98	97
22							1 5	68	14	4 8	3 3	83	70

stable in a few children (nos 16 20) Cramps and cardiovacular collapse both due to excessive ultrafiltration were easily corrected by saline perfusion Hard water syndrome with headaches vomiting and hypercalcemia due to defective functioning of the water softener occurred on a few occasions

Arterial blood pressure

With removal of fluid by ultrafiltration, arterial pressure returned to normal in all children except 5 in one case (no 18) arterial pressure is controlled by high doses of antihypertensive drugs In 3 other patients (nos 7 12, 21) malignant hypertension compelled us to binephrectomy Binephrectomy led to normalization of arterial pressure within a few hours and regression of cachexia within a few weeks In the last patient (no 10) hypertension was partially controlled by medical treatment but cardiac volume progressively increased during the first 18 months of dialysis This led to binephrectomy with subsequent correction of cardiovascular symptoms

Anemia

Transfusions were given in order to maintain an hematocrit level over 15 ° High transfusion requirements were more frequently observed after binephrectomy (2 out of 4 children) but were also seen in other patients (3 out of 21) Transfusion requirements were correlated neither to duration of dialysis nor to quality of epuration

Bone

Two situations can be distinguished according to the presence or absence of osteodystrophy at the onset of dialysis

1 Bone X Rays and the level of alkaline phosphatases were normal at the onset of dialysis in 20 out of 25 children Only 11 of these have been followed for more than 1 year In 10 of these 11 cases the radiological aspect of bone is not modified after 12 to 32 months of dialysis with calcium and vitamin D2 supple

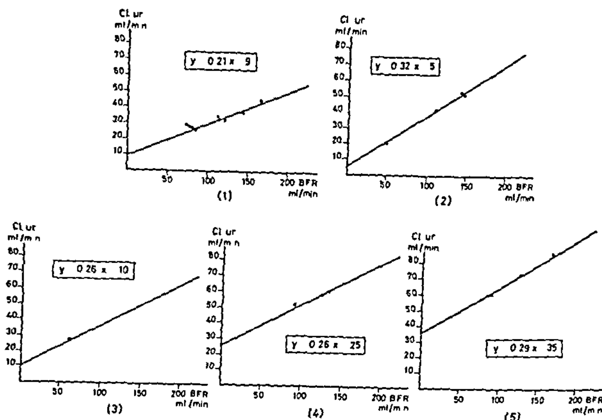


Fig 2 Comparison of clearance of urea (*Cl ur*) in three types of dialyser according to blood flow rate (*B F R*) 1) Kiil one layer 2) Pediatric Kiil two layers 3) Rhone Poulenc four layers 4) Rhone Poulenc six layers 5) Rhone Poulenc eight layers

mentation (4 000 to 12 000 U/day) In one child however (no 12), severe radiological osteodystrophy with increased alkaline phosphatases has appeared after 9 months of dialysis in spite of administration of 6 000 U/day of vitamin D2

It is now well established that frequency of bone disease increases with the duration of regular dialysis to reach 20 to 50% after 1 year Systematic vitamin D2 and calcium supplementation can prevent this complication as shown in this series of patients But no standard treatment can be delineated In some cases doses may be insufficient particularly if growth continues during dialysis This was observed in case 12 who had a mean growth of 5 6 cm per year On the other hand vitamin D2 or 25 OH cholecalciferol may produce hypercalcemia (see later, case 14) and/or vascular calcifications In fact, no arterial calcifications have appeared in our patients, but regular ophthalmoscopic controls have shown corneal calcifications in 4 cases (nos 5, 13, 18 20) after 3 to 20 months of vitamin treatment

2 Five children with renal failure of long standing and no or inadequate vitamin D2 treatment presented with osteodystrophy of varying severity before the inception of dialysis Under regular dialysis and Vitamin D therapy the evolution of bone lesions was variable and unforeseeable dramatic improvement was noted in one girl (case 14) she had severe osteodystrophy with bilateral fractures of the femoral neck and received at first 16 000 U/day of vitamin D2 for 10 months without radiological modification After 2 months treatment with 16 000 U/day of 25 OH cholecalciferol there was obvious improvement of radiological signs including subperiostal resorption A bone biopsy showed regression of fibrosis and decrease of osteoid volume but severe hypercalcemia occurred which compelled to parathyroidectomy Partial but significant and persistent improvement was noted in three other cases (cases 19, 20 22) with lower doses of 25 OH cholecalciferol (1 000

to 6 000 U/day) Mild hypercalcemia occurred in two of these cases but disappeared 1 week after the arrest of treatment Bone lesions were not modified in another case (no 16) after 8 months of vitamin D2 (10 000 U/day) and 4 months of 25 OH cholecalciferol (2 100 U/day)

In conclusion it seems that vitamin D therapy is useful in dialysed children for prevention and cure of osteodystrophy But the vitamin requirement is different in each case and cannot be routinely evaluated because study of calcium intestinal absorption is too long and tedious Therefore one is bound to rely on biochemical and radiological symptoms although their evolution is too slow to allow treatment to be accurately adjusted The usefulness of aluminium hydroxide is more difficult to assess but adequate doses lower phosphate levels and in 1 case (no 4) severe pruritus disappeared simultaneously

Growth

Growth is generally impaired in hemodialysed children though to a variable degree Further data on this subject will be published separately (2)

Other complications

During this 290 patient month experience few complications have occurred Two pericarditis regressed after increasing dialysis time One patient survived severe hyperkalemia (9 3 mEq/l) with cardiac arrest by resuscitation and emergency dialysis Four episodes of pulmonary oedema were ascribed to insufficient ultrafiltration This outlines the difficulty of estimating fluid overload especially in young growing children

Biological symptoms of hepatitis were found in 4 children and Australian antigen was present in blood in 7 cases Four members of the staff have had moderately severe clinical and biological hepatitis

Among neurological complications no polyneuritis was observed regular studies of electromyograms and of nervous conduction speed have shown no major alteration But seizures repeatedly occurred in 2 children (nos 12 and 14) during hemodialysis Both had persistent electroencephalographic anomalies between dialysis sessions In 1 case (no 12) seizures disappeared with continuous phenobarbital treatment and the electroencephalogram has reverted to normal In the other case (no 14) seizures became permanent leading to coma and sublimal exploration showed cerebral oedema The child died 5 months later of intracranial hemorrhage due to anticoagulant treatment

Psychological aspect

Adaptation appears generally good if one relies on superficial observation of children s behaviour Physical and mental relief is such that a fairly normal life is rapidly resumed and material problems brushed aside However these may be important four families (nos 3 10 16 and 25) had to move to Paris and to find new homes and jobs and 4 children are separated from their families (nos 4 8 19 and 20) No major psychiatric problems have appeared About half of the older children seem to compensate their health problems and possibly their anxiety by school performance (among these are 2 girls nos 10 and 16 who had never been to school before) and by participation in their treatment Best acceptance appears to be in the youngest children (nos 12, 19 and 20) although one may expect interference with the development of their personalities insofar as illness and treatment has led to parental overprotection in case 12 and to separation from their families in cases 19 and 20 In about one third of children withdrawal and lack of communication with nurses and doctors probably corresponds to another defence mechanism

More precise appraisal of psychological status through projective testing and interviews (8) has shown however clear alterations of personality development in almost all cases

Organizational features

Although there is no deep technical difference between regular dialysis in children and adults it is obviously desirable that pediatricians should continue to care for children with terminal uremia By their training, pediatricians seem more able to cope with the triangular relationship between doctors, children and parents and to help children to attain a better rehabilitation Furthermore, problems of growth pubertal maturation and osteodystrophy are directly in the field of pediatrics *Therefore the principle of specifically pediatric* centres appears to be a good solution for treatment of children with terminal uremia

On the other hand it is obviously impossible to create pediatric units everywhere, if one considers the cost of such an investment Frequency of terminal uremia in children has not been accurately determined figures of 1 to 2 per million and per year have been given but they are probably an underevaluation (6 10) On the basis of this information it seems reasonable to aim for the creation of a pediatric centre in an urban area of at least 3 to 5 million people Such centres already exist in a few cities But whenever it is impossible for various reasons to set up a pediatric unit a pediatric area could be included in a standard dialysis center There pediatricians could follow their patients with the collaboration of nephrologists Such a solution would allow easier and better treatment for children with terminal uremia wherever a dialysis centre exists (5 9)

SUMMARY

Regular hemodialysis has been performed in 25 children and adolescents 20 months to 20 years old for periods up to 2 years The eight layer Rhone Poulenc dialyser has appeared well adapted to children, as it allows of the possibility of reducing extracorporeal blood volume to less than 100 ml and to modify it according to weight *Long term* use of Scribner shunts has proved difficult Subcutaneous arteriovenous fistulas seem a better solution at present, even in young children In children under 8–10 kg difficulties concerning access to vessels remain a limitation on regular dialysis Clinical biochemical results and psychological tolerance have been satisfactory The validity of regular hemodialysis in children in view of kidney transplantation now appears unquestionable

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DEAD SPACE REBREATHING OF AIR AND OXYGEN IN CHILDREN

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The stimulatory effect of CO inhalation on human respiration is well known (1 2 4 5 6 7 8 9 10) While this has been studied extensively in adults there is to our knowledge only one study pertaining to children and this was done in newborn infants (1) Data on children beyond the infant period are com pletely lacking

There are several methods of administering CO to awake human subjects

1 CO may be supplied via a tube and face mask from a gas tank containing CO in air

2 Endogenously produced CO may be re breathed by artificially increasing the res piratory dead space (6 7 8 10) This may be done (*a*) in a closed system by letting the subject rebreathe from a bag containing pure oxygen By doing so a gradual built up of CO within the inspiratory and expiratory air mixture will be achieved thus producing an increasing stimulation to respiration (*b*) an open-ended tube may be placed in front of the subject s mouth and nose forcing the subject to inspire previously exhaled alveolar air but at the same time making it possible for the subject to take in room air at the end of inspiration This latter method was chosen in the present investigation

It was known from rebreathing studies in adult subjects (7) that generally most sub- jects will reach a steady state pattern of venti lation after a brief period of hunting pro vided the size of the dead space tube is chosen appropriately The obvious advantage of this method is the simplicity of the experimental set up and the supposedly even breath to breath stimulation due to CO

The aim of the study was twofold 1 to provide the data for the missing link be- tween infants and adults, i e CO-ventilation relationships in children beyond the infant period and thus be able to compare the CO sensitivity of children with that known to exist in infants and adults 2 to establish an approximate relationship in children of the increase in ventilation and the size of dead space tube needed to achieve a certain aug mentation of respiration This relationship may be relevant in the prophylaxis and ther- apy of pulmonary atelectasis where the method of dead space rebreathing has shown its usefulness in adult patients for many years (10)

MATERIALS AND METHODS

22 pediatric patients without signs of cardio pulmo nary disease ranging in age from 4 1/12 to 14 3/12 years were studied The patients were divided into two groups In group I (12 patients) the subj cts re brea hed room air in group II (10 patients) the sub jects rebreathed pure oxygen Pertinent vital and clini cal data are given in Table 1 for group I and in Table 2 for group II

The subjects were selected at random from the population of our general pediatric hospital The only requirements for entry into the study were (*a*) an age of more than 4 years because of the cooperation required for the test (*b*) the absence of any cardio-

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Table 1. *Vital and clinical data on 12 subjects (group I)*

The patients were selected at random, the only prerequisites for entry into the study being (1) an age of more than four years, (2) the absence of cardio-pulmonary disease

No	Age (y)	Sex	Body weight (kg)	Height (cm)	Diagnosis
1	4 1/2	F	23.0	115	Uveitis
2	6	F	20.6	123	Renal disease
3	8	F	24.5	135	Renal disease
4	12	F	45.0	157	Headache of unknown origin
5	4	F	14.5	109	Urinary tract infection
6	5	M	16.3	114	Convulsive disorder
7	9	M	30.0	?	Convulsive disorder
8	12	F	67.4	155	Obesity
9	10	M	29.6	153	Acid ingestion
10	7	M	17.9	106	Growth retardation
11	14	M	46.2	168	Convulsive disorder? Lymph node disease
12	12	M	65.3	150	Obesity

pulmonary disease. The subjects were comfortably seated in a chair and the purpose of the experiment was explained to them. In particular, it was stated that no pain would be connected with the study. The younger children were given toys, the older children were provided with comic strips in order for them to pay as little attention to the laboratory environment as possible.

The rebreathing equipment consisted of a face mask (either Dräger No. 2 or Everseal No. 3, depending on the size of the child) to which a respiratory flow meter type GM 577 was attached via a small plastic connecting tube having an internal diameter of 17 mm. This arrangement will be referred to as the "basic apparatus"; the ventilation achieved when this was used as "resting ventilation". The dead space of the "basic apparatus" varied slightly with the type of mask but was approximately 145 ml. At the distal end of it plastic tubes (internal diameter 17 mm) having a volume of 100, 200, 300 or 400 ml respectively were attached. In the group I experiments (air rebreathing) these were left open-ended. In the group II experiments (O_2 rebreathing) a short metal connector was inserted at the end leading into a large rubber balloon (37 L) which had previously been filled with pure oxygen.

The respiratory flow meter of the basic apparatus could be heated yielding a temperature of inspired air of 34°C. It was connected to a pneumotachograph Godart GM 577 (range of air flow 0–100 liters per minute). Through the small connecting tube between the face mask and the flow meter a needle was advanced to the center of the lumen through which respiratory air could be suctioned to an infrared CO_2 analyser (URAS 4, Hartmann & Braun) at a rate of 300 to 500 ml per minute. A polarographic O_2 electrode was built into the infrared CO_2 analyser just distal to the chamber according to the recommendations of Beneken Kolmer & Kreuzer (3) for instantaneous O_2 analysis of respiratory air. This arrangement enabled us to analyze the respiratory gas for CO_2 and O_2 simultaneously. All signals including the integration of the respiratory flow curve of the pneumotachograph were connected to the appropriate amplifiers of a DR 8 recorder (Electronics for Medicine), displayed on an oscilloscope and recorded on photographic paper.

In the group II experiments the O_2 balloon was frequently refilled with pure oxygen in order to avoid CO_2 accumulation. Thus for the purpose of the experiment the group II arrangement will also be considered "open-ended", the large balloon being regarded as an infinite reservoir of pure oxygen just as room air in the group I experiments.

Course of experiments

After appropriate adjustment time the basic apparatus was tightly attached over the subject's nose and mouth. Frequently an initial hyperventilation was observed during which no recording was done. When we thought the subject to be in a reasonable steady state all signals were recorded without the child's knowledge. This recording was subsequently considered the subject's resting ventilation. After a five minute rest during which all instruments were recalibrated, the subject was again asked to breathe through the basic apparatus. This time an additional dead space of 100 ml was attached. The dead space

Table 2. *Same data as given in Table 1 for group 2 (O_2 rebreathing)*

For details see legend of Table 1

No	Age (y)	Sex	Body weight (kg)	Height (cm)	Diagnosis
1	12	F	33.0	147	Mental retardation
2	8	F	31.9	137	Urinary tract infection
3	6	F	26.0	126	Diabetes mellitus
4	12	M	40.0	140	Eosinophilic granuloma
5	8	M	26.0	?	Convulsive disorder
6	14	F	45.0	158	Enteritis
7	12	M	31.6	151	Convulsive disorder, goiter
8	12	M	43.6	158	Diabetes mellitus
9	9	F	25.0	132	Facial palsy
10	6	F	31.0	125	Urinary tract infection

olume was subsequently increased in 100 ml steps a total of 400 ml

In the youn er patients the increase was only armed to 200 or 300 ml the maximum additional lead space volume being determined by the respira ory CO_2 pattern As soon as CO failed to return to zero during the inspiratory phase the experiment was discontinued and the previous dead space volume was considered the upper limit for that particular child.

None of the experimental subjects actually com plained of any discomfort nor did they develop any symptoms other than the expected hyperventilation In particular there was no cyanosis Though the chil dren were quite apprehensive the first time they came to the laboratory they always lost their anxiety and were happy to volunte r when they were called again

Calib ations

The pneumotachograph was calibrated using a volu meter (No 651 Drager) through which air was passed at a known flow Expiratory and inspiratory flow was integrat d separately yielding tidal vol umes. Expiratory volumes were used for calculation of ventilation The CO analyser was calibrated elec trically the electrical calibration being checked pe riodically with known concentrations of CO CO concentrations were converted into partial pressure using corrected barometric pressure for calculation

The O electrode was calibrated for each experi ment using 100% O and air as calibrating gases All results are expressed in A T P S

A total of 88 measurements were performed in the subj cts Statistical evaluation was done using standard methods of calculating mean valu s ± stand ard deviation In estimating the significance of dif ferences between different sets of values the Student s t-test was applied Regression lines were cal ulated using the formula of paired values for analysis of variance

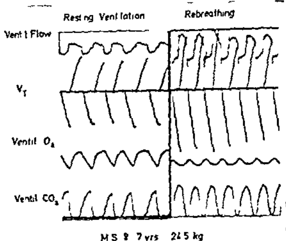


Fig 1 Original tracing of girl M S 7 years old 24.5 kg Air rebreathing From top to bottom (1) Respiratory flow curve recorded by pneumotacho-graph (2) integrals of individual tidal volumes separated into inspiratory and expiratory phase (3) respiratory O curve (4) respiratory CO curve On the left hand side resting ventilation is shown with the basic apparatus applied (145 ml) On the right hand side an additional 200 ml tube has been given mak ing up a total additional dead space of 345 ml. The increase in ventilation is demonstrated by the in crease in tidal volume and a slight rise in respiratory frequency The respiratory O_2 pattern is characterized by a lowering of inspiratory P_O while alveolar P_O has remained unchanged There is a marked increase in alveolar P_{CO} During inspiration the CO_2 line always reaches zero indicating that room air is in haled in addition to the dead space volume respired

RESULTS

The results will be reported according to the two sets of experiments as previously men tioned Figs 2 and 6 and Tables 1 and 3 are related to the group I experiments i e re breathing room air Tables 2 and 4 on the other hand were obtained from the group-II experiments i e O rebreathing

Fig 1 shows a typical tracing of an experi ment On the left side the subject is breathing quietly through the basic apparatus (V_D 145 ml) The upper trace shows the respiratory flow curve with the simultaneous integration of inspiratory and expiratory flow just below yielding tidal volume (V_T) The lowest tracing is that of respiratory CO second from bottom that of respiratory O On the right the patient is breathing through an additional dead space of 200 ml thus the total additional dead space is now 345 ml There is considerable increase in ventilation as manifested by the increase in tidal volume as well as in respira tory frequency Alveolar P_{CO} is considerably higher than it was during resting ventilation but there is a return to the zero line during each inspiratory phase documenting the in halation of room air in addition to the re breathed alveolar air from the dead space The O tracing shows a marked decrease in the difference between inspiratory and expira-tory P_O This is exclusively due to the reduc

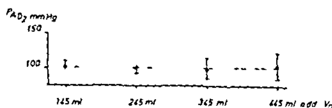


Fig 2 Alveolar O_2 tension (ordinate) plotted over added dead space in subjects rebreathing room air There is no change in mean alveolar O_2 tension with increasing dead space in the air breathing situation This indicates that up to a dead space of 445 ml hypoxia is not likely to occur with air rebreathing

tion in inspiratory O which in turn is certainly a result of mixing of alveolar and room air which the patient inhales Interestingly the alveolar P_{O_2} has remained unchanged suggesting a normal arterial P_O at this level of ventilation

This latter point is further supported by Fig 2 in which mean alveolar O tensions ± 1 S D) are listed in relation to the volume of dead space added It is evident that the mean alveolar oxygen tension does not change significantly even when rather large dead space volumes are used Thus an additional hypoxic drive is unlikely to be superimposed on the CO_2 stimulation

On the other hand there is a fair increase in mean alveolar P_{CO_2} with increasing dead space (Fig 3) This is true for both the air rebreathers and the O_2 rebreathers Interestingly the mean alveolar P_{CO} at the beginning of the experiments was usually quite low demonstrating a fair degree of hyperventila

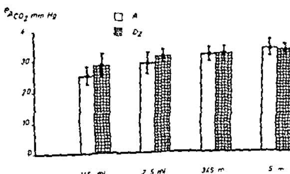


Fig 3 With increasing added dead space (abscissa) there is a continuous rise in alveolar CO tension The amount of rise is practically identical in air rebreathing compared to O rebreathing It is noteworthy that the subjects were usually hyperventilating at the beginning of the experiment as is evident by the low alveolar P_{CO} at the onset of the experiment (left columns) Even with large volume of added dead space CO tension rarely exceeds 40 mmHg

Table 3 *Results of group 1 experiments (air rebreathing)*

The numbers correspond to those used in Table 1 Columns (from left to right) (1) order of subjects (2) volume of added space (add V_D) (3) tidal volume (V_T) (4) respiratory frequency (f) (5) minute ventilation ($V_T \times f$) (6) alveolar CO_2 tension (P_{ACO_2})

No	Added V_D (ml)	V_T (ml)	f (min^{-1})	V_E (L/min)	P_{ACO} (mm Hg)
1	145	430	26	11 14	22 4
	245	300	31	9 31	25 6
	345	460	38	17 49	30 4
	445	890	35	31 24	32 0
2	145	260	20	5 23	24 0
	245	420	22	9 34	28 8
	345	430	23	10 10	28 8
	445	710	21	15 21	30 4
3	145	280	19	5 31	27 2
	245	270	24	6 37	32 0
	345	660	24	15 99	33 6
	445	790	21	16 65	36 8
4	145	260	20	5 30	25 3
	245	280	20	5 77	26 9
	345	820	23	19 16	34 9
	445	970	20	19 45	38 0
5	145	120	28	3 61	21 5
	245	180	30	5 57	24 0
	345	520	34	17 95	31 5
	445	450	36	16 44	30 0
6	145	180	19	3 47	26 9
	245	250	18	4 55	31 2
	345	450	22	9 94	34 1
	445	530	25	13 40	34 1
7	145	100	24	2 59	29 8
	245	150	26	4 10	31 2
	345	250	31	7 85	34 1
	445	600	27	16 25	39 3
8	145	530	21	11 22	27 9
	245	800	21	16 92	30 8
	345	1 260	23	29 14	33 6
	445	1 400	22	30 85	34 9
9	145	220	33	7 47	21 1
	245	280	28	8 05	29 6
	345	950	19	16 24	32 4
	445	1 270	28	45 70	36 6
10	145	250	22	5 63	25 3
	245	580	27	15 82	28 2
	345	930	30	28 16	30 9
	445	1 460	29	42 48	33 8
11	145	460	23	10 73	30 6
	245	790	21	16 75	38 6
	345	1 200	21	25 36	36 3
	445	1 920	18	33 19	37 8
12	145	280	25	7 20	23 4
	245	530	23	12 37	27 3
	345	670	27	18 34	28 6
	445	970	26	25 35	29 9

tion due to apprehension The amount of rise is approximately equal in both sets of experiments

All single values of minute ventilation (V_E) and alveolar CO_2 tension ($P_{A_{CO_2}}$) are shown in Tables 3 and 4 It is clear from the values that with increasing dead space a rise of ventilation could always be achieved

On the other hand there is substantial variation in the individual degree of CO_2 tolerance as shown by the figures on alveolar CO_2 tension Fig 4 shows a graphical representation of the classical CO_2 response curve of minute ventilation plotted over alveolar CO_2 As would be expected from the results known from adult subjects the scatter is quite considerable However calculation of the regression line shows a highly statistically significant correlation in both sets of experiments with the slopes of the two regression lines of air rebreathers and O_2 rebreathers being almost identical The slight upward displacement of the line in the O_2 experiments is probably due to a slightly greater resistance imposed in this set of experiments because of the additional metal connector leading to the O_2 balloon The increase in minute ventilation was primarily due to an increase in tidal volume This is demonstrated in Fig 5 by the minimal change observed in respiratory frequency when the different dead space volumes are used Interestingly the O_2 rebreathers showed a greater increase in frequency than air rebreathers

In order to look at the results from the point of view of tube sizes for individual patients in order to achieve a certain increase in ventilation the plot of Fig 6 was drawn Ventilation is expressed as ventilation ratio (VR) fold increase in ventilation plotted over dead space volume per kg body weight As can be seen there is great variation in individual values principally due to the difference in individual CO_2 tolerance Nevertheless a highly significant regression line could be calculated which may be used as a guide line if the tubes are to be used for therapeutic purpose

Table 4 *Results of group 2 experiments* (O_2 *rebreathing*)

The numbers refer to the same individuals as in Table 2 For more details see legend of Table 3

No	added V_D (ml)	V_T (ml)	f (min^{-1})	V_E (L/min)	$P_{A_{CO_2}}$ (mmHg)
1	145	260	25	6 70	22 35
	245	930	36	34 59	29 80
	345	860	31	27 29	32 78
	445	1 000	32	32.11	32 78
2	145	690	20	13 84	28 31
	245	870	23	20 04	31.29
	345	940	29	27 44	31 29
	445	1 010	27	27.27	35 76
3	145	1 180	21	24 89	29 80
	245	960	27	25 91	32 78
	345	910	27	24 67	32 78
	445	1 070	29	31 19	35 76
4	145	660	22	14 68	27 20
	245	810	27	22.01	30 40
	345	890	28	25 04	33 60
	445	1 150	26	29 97	35 20
5	145	1 070	22	20 44	33 60
	245	970	27	27 28	35 20
	345	960	28	25 19	36 40
	445	910	26	27 43	36 40
6	145	760	25	19 03	32 97
	245	770	28	21 77	32 97
	345	900	28	25 27	32 97
	445	840	30	25 28	32 97
7	145	340	28	9 63	23 94
	245	310	30	9 39	29 57
	345	910	34	31 02	29 57
	445	830	40	33 41	30 98
8	145	1 140	19	21 77	34 50
	245	940	23	21 67	35 70
	345	1 050	27	28 46	35 20
	445	900	27	33 63	36 61
9	145	670	25	16 81	29 57
	245	520	26	13 52	30 98
	345	1 090	24	26 35	33 79
	445	940	30	25 41	35 20
10	145	460	28	12 96	23 76
	245	420	31	13 13	27 72
	345	810	30	24 34	27 72
	445	1 260	31	39 17	30 36

DISCUSSION

These results obtained in a pediatric population confirm the notion about CO_2 response of ventilation in adults (1 2 4 5 6 7 8, 10) A regular increase in ventilation is observed when CO_2 is given to breathe It is also known from adults that in most individuals minute ventilation increases primarily on account of tidal volume with little change in respiratory frequency (10) The comparison between air rebreathing and O_2 rebreathing shows prac

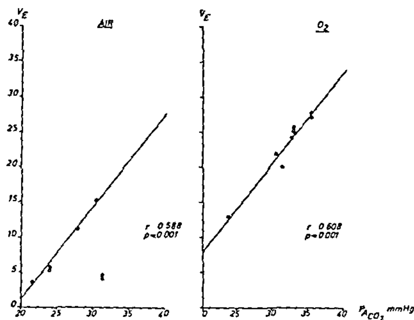


Fig 4 CO response curves (VE plotted over alveolar CO_2 tension) in air re breathing (left) versus O_2 rebreathing (right) In comparison the O curve is somewhat displaced upward to the left, probably as a result of the slightly greater respiratory resistance of the re breathing system imposed by the metal connector to the O_2 containing balloon It is intriguing to note that th slopes of both regression lines are almost identical indicating that the CO_2 r sponse is the only determinant in the ventilatory response of rebreathing

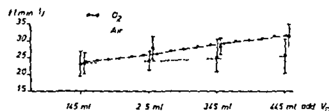


Fig 5 Respiratory frequency (*f*) shows little change with increasing dead space volume There is a slight difference between O and air rebreathing in this re spect in O_2 rebreathing the increase in frequency is more pronounced In general the ventilatory increase observed in dead space rebreathing is almost entirely due to an increase in tidal volume

tically no difference This is in agreement with previous experiments in adults by Schwartz et al (10), who found a slightly greater in crease of ventilation in O_2 rebreathers as compared to air rebreathers Additional in formation regarding this particular problem is furnished by the registration of respiratory O_2 (Fig 2) demonstrating that within the limits used in the application of dead space volume in the present experiments, no hypoxia occurred

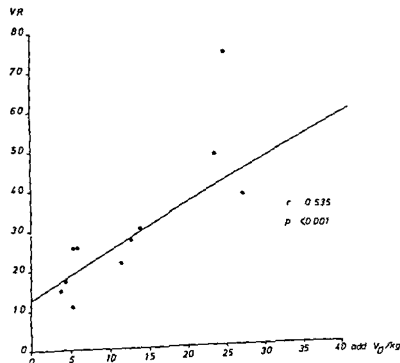


Fig 6 Ventilation ratio plotted against added dead space per kg body weight Individual values are indicated by the black dots There is great variation The line drawn is the calculated regression line As indicated by the numbers (*r* and *p*) there is a highly statistically signifi cant correlation The results are from the air rebreathing experiments only The correlation indicates that addi tional dead space volume (in ml) of just below three times the individuals body weight (in kg) is necessary for an ap proximately two fold increase over rest ing ventilation The relatively wide scatter in individual results is due to the difference in CO_2 sensitivity among dif ferent individuals

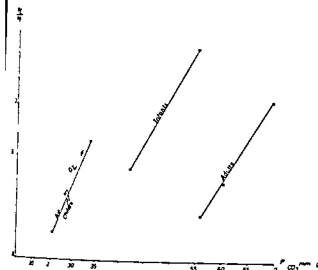


Fig 7 CO response curves in our subjects (2 curves on the left) in comparison with results reported in the literature on infants and adults by Avery et al (1) As can be seen the slopes of all four are almost identical The results in our children are shifted to the left due to th low alveolar CO tension observed in our subjects The similarity in slope signifies the similarity in CO sensitivity at any period of life

In order to arrive at a statement about the CO sensitivity in our children as compared with adults and infants reported in the litera ture we recalculated the data by Avery et al (1) obtained from infants and adults to fit a plot of minute ventilation per kg body weight ($\dot{V}_E$/kg) over alveolar CO_2 tension ($P_{A_{CO}}$) (Fig 7) The figure shows an almost perfect agreement of all our results with those of Avery et al with regard to the slope of the response curve There is however a curious displacement of the whole response curve of our children to the left due to the very low alveolar CO values we were dealing with As was mentioned before this must be due to the fact that the children were quite apprehensive and always hyperventilating at the beginning of the experiments

Up to this point the therapeutic aspects of the dead space rebreathing tube seem to have not been properly evaluated in children It appears reasonable to believe that it could prove useful particularly in the prevention of atelectasis in those children who do not co-operate in taking deep breaths This would apply primarily to patients having undergone surgery A controlled study will be attempted in the future to evaluate the quality of the rebreathing tube in these clinical situations

SUMMARY

Using an open rebreathing method CO response curves were obtained in children of kindergarten and school age Air and O were given to respire the volume of added ventilatory dead space was increased stepwise and stopped when inspiratory CO failed to reach zero The slope of the $\dot{V}$/CO relationships in our subjects was in good agreement with those reported in the literature in adults and in infants when corrections were made for the difference in body size The difference between O rebreathers and air rebreathers was negligible

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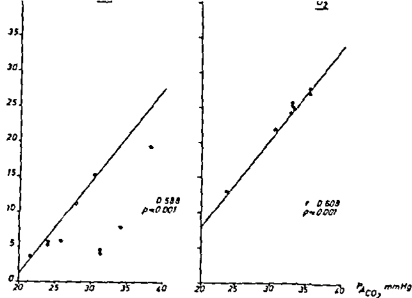


Fig 4 CO_2 response curves (V_E plotted over alveolar CO_2 tension) in air re breathing (left) versus O_2 rebreathing (right) In comparison the O curve is somewhat displaced upward to the left, probably as a result of the slightly greater respiratory resistance of the re breathing system imposed by the metal connector to the O_2 containing balloon It is intriguing to note that th slopes of both regression lines are almost identical indicating that the CO_2 re sponse is the only determinant in the ventilatory response of rebreathing

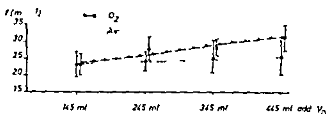


Fig 5 Respiratory frequency (*f*) shows little change with increasing dead space volume There is a slight difference between O and air rebreathing in this re spect in O_2 rebreathing the increase in frequency is more pronounced In general the ventilatory increase observed in dead space rebreathing is almost entirely due to an increase in tidal volume

tically no difference This is in agreement with previous experiments in adults by Schwartz et al (10), who found a slightly greater in crease of ventilation in O_2 rebreathers as compared to air rebreathers Additional in formation regarding this particular problem is furnished by the registration of respiratory O_2 (Fig 2) demonstrating that within the limits used in the application of dead space volume in the present experiments, no hypoxia occurred

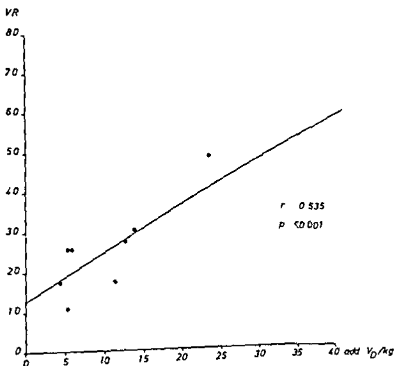


Fig 6 Ventilation ratio plotted against added dead space per kg body weight Individual values are indicated by the black dots There is great variation The line drawn is the calculated regression line As indicated by the numbers (*r* and *p*) there is a highly statistically signifi cant correlation The results are from the air rebreathing experiments only The correlation indicates that addi tional dead space volume (in ml) of just below three times the individuals body weight (in kg) is necessary for an ap proximately two-fold increase over rest ing ventilation The relatively wide scatter in individual results is due to the difference in CO sensitivity among dif ferent individuals

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INFLUENCE OF PHOTOTHERAPY ON UNCONJUGATED BILIRUBIN IN DUODENAL BILE OF NEWBORN INFANTS WITH HYPERBILIRUBINEMIA

A Preliminary Study

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From the Department of Paediatrics Copenhagen County Hospital Glostrup and the Institute of Medical Biochemistry University of Aarhus Denmark

In 1958 Cremer et al published the first report of phototherapy in newborn infants with hyperbilirubinemia (3) Since then several investigations have confirmed the effect of this treatment (5 6 8 10 11) No serious side effects of phototherapy have been observed However very little is known as regards the biochemical processes involved in the photodestruction of bilirubin in the early postnatal period Experiments with ^{14}C bilirubin in Gunn rats with bile fistula have shown an increased excretion of unconjugated bilirubin and water soluble breakdown products of bilirubin during phototherapy (9) In 2 infants 5 and 7 months old suffering from Crigler Najjar s disease a similar investigation was performed and the ^{14}C activity was recovered in duodenal bile as well as bilirubin as in water soluble breakdown products (2)

It is a constant observation that after a few hours of phototherapy the stools of the infants become loose and turn to a greenish lo[illegible] t be [illegible]ue to increased bili[illegible] former ex[illegible] n point to [illegible]retion of [illegible] ed with [illegible]ilirubin in the duodenal bile of newborn infants subjected to phototherapy

MATERIAL AND METHODS

The material comprises 12 newborn infants with serum bilirubin concentration ≥100 mg/l without signs of isoimmunization Birthweight ≥2 000 g Five hours after the last meal which consisted of 10% glucose a thin rubber catheter in a one step procedure was introduced through the nostril into the lower part of the descendent duodenum Fluoroscopy confirmed the position of the catheter Over the next 1–2 hours a sample of duodenal bile could be collected Phototherapy (100 footcandles light unit equipped with 8 white 20 Watt fluorescent bulbs) was given for the following 24 hours in 7 patients, while 5 patients received no therapy and served as controls At the end of this period a second bile sample was collected In both samples an analysis of the concentration of unconjugated bilirubin dry weight substance and phospholipid was performed Unconjugated bilirubin was determined by the chloroform extraction method (1)

RESULTS

A survey of the 12 patients is given in Table 1 Figs 1 and 2 show serum bilirubin levels in the individual patients in the 24-hour intervals before the first bile sample between the 2 samples and after the second bile sample During phototherapy a profound change in the appearance of duodenal bile takes place

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It is a constant observation that after a few hours of phototherapy the stools of the infants become loose and turn to a greenish colour This might be due to increased biliverdin formation in the gut The former experiments and the latter observation point to the bile as the primary site of excretion of photo-oxidation products of bilirubin

This preliminary study is concerned with the concentration of unconjugated bilirubin in the duodenal bile of newborn infants subjected to phototherapy

MATERIAL AND METHODS

The material comprises 12 newborn infants with serum bilirubin concentration ≥100 mg/l without signs of isoimmunization Birthweight ≥2 000 g Five hours after th last meal which consisted of 10% glucose a thin rubber catheter in a one step procedure was introduced through the nostril into the lower part of the descendent duodenum Fluoroscopy confirmed the position of the cath ter Over the next 1–2 hours a sample of duodenal bile could be collected Phototherapy (100 footcandles light unit, equipped with 8 white 20 Watt fluorescent bulbs) was given for the following 24 hours in 7 patients, while 5 patients received no therapy and served as controls At the end of this period a econd bile sample was collected In both samples an analysis of the concentration of unconjugated bilirubin, dry weight substance and phospholipid was performed Unconjugated bilirubin was determined by th chloroform extraction method (1)

RESULTS

A survey of the 12 patients is given in Table 1 Figs 1 and 2 show serum bilirubin levels in the individual patients in the 24-hour intervals before the first bile sample between the 2 samples and after the second bile sample During phototherapy a profound change in the appearance of duodenal bile takes place

Table 1 *Survey of 12 newborn infants, in whom duodenal bile samples were collected*

Pat no	Sex	Birthweight (g)
Phototherapy (n = 7)		
1	o	2 250
2	♂	2 450
3	♂	3 050
4	♂	3 700
5	o	2 600
6	♂	3 250
7	♀	2 200
Control (n = 5)		
1	♀	2 400
2	♀	2 270
3	♂	2 800
4	♀	2 000
5	♀	2 150

From a pure yellow, the colour turns brownish black. From Table 2 it is seen that the mean concentration of unconjugated bilirubin in duodenal bile is approximately doubled

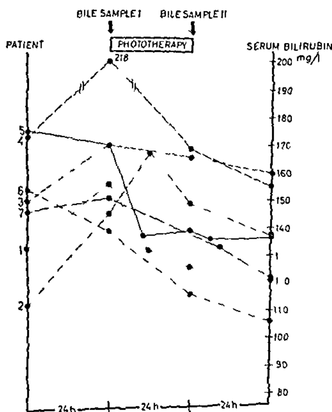


Fig 1 Serum bilirubin concentrations in 7 patients in the 24 hour intervals before the first duodenal bile sample between the first and second duodenal bile sample and after the second duodenal bile sample. Phototherapy was given between bile sample I and bile sample II

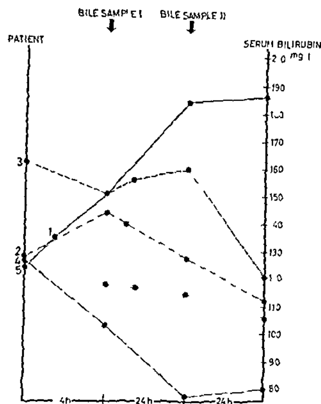


Fig 2 Serum bilirubin concentrations in 5 control patients in the 24 hour intervals before the first duodenal bile sample between the first and second duodenal bile sample and after the second duodenal bile sample

during phototherapy. Compared with the changes of the concentration of unconjugated bilirubin in the patients during phototherapy the changes observed in the control patients are negligible. The difference of the means is statistically significant ($p = 0.05$). Patient no 4 had a much higher bile concentration of unconjugated bilirubin than the rest of the patients receiving phototherapy. This patient also had the highest serum bilirubin concentration (Fig 1).

In an effort to estimate potential changes in bile flow during phototherapy an analysis of the concentration of dry weight substance and phospholipid was carried out. This was done assuming that the 24 hour excretion in bile of as well dry weight substance as phospholipid was relatively constant (4) under normal conditions. The results of these analyses are given in Table 3. From this it is seen that in the individual patient the concentration of dry

weight substance seems more constant than the concentration of phospholipid so the concentration of the former seems a better choice than the concentration of phospholipid as an indicator of bile flow changes. However in this preliminary study the number of analyses are too few and the figures too scattered to form a basis of such an estimation.

DISCUSSION

This investigation has shown that phototherapy increases the *concentration* of unconjugated bilirubin in duodenal bile. After 24 hours the mean concentration approximately increased twofold. As we do not know if alterations in bile flow have occurred during phototherapy we cannot with certainty tell that a similar augmentation of unconjugated bilirubin *excretion* in bile has been effected. If however no increase in the excretion has occurred one must suppose that the duodenal bile flow on the average has been halved during phototherapy. This assumption does not correspond with the tenfold increase in *excretion* of unconjugated bilirubin during phototherapy found by Ostrow in his experiments with Gunn rats with bile fistula (9).

Table 2. *Concentration of unconjugated (µmol/l) bilirubin in duodenal bile in 7 patients receiving phototherapy and in 5 control patients*

Bile samples I and II collected with an interval of 24 hours

Pat no	Bile sample I	Bile sample II
Phototherapy (n = 7)		
1	23	33
2	14	42
3	23	30
4	60	127
5	13	35
6	21	19
7	21	43
Mean	25	47
Control (n = 5)		
1	17	20
2	9	12
3	47	10
4	9	12
5	19	20
Mean	19	15

Table 3. *Concentration of dry weight substance and phospholipid in duodenal bile in 7 patients receiving phototherapy and in 5 control patients*

Bile samples I and II collected with an interval of 24 hours

Pat no	Dry weight substance (mg/100 µl) Bile sample I	II	Phospholipid (mmol/l) Bile sample I	II
Phototherapy (n = 7)				
1	—	—	0.15	1.07
2	—	—	0.85	1.39
3	2.1	1.7	0.77	0.70
4	3.5	3.3	1.80	1.60
5	—	—	0.91	1.00
6	2.6	2.5	1.99	1.75
7	2.2	2.3	1.89	1.30
Mean	2.6	2.5	1.19	1.26
Control (n = 5)				
1	2.0	1.6	2.17	1.22
2	2.0	3.6	1.93	1.85
3	1.8	1.7	1.12	0.71
4	2.1	1.9	0.28	0.16
5	2.3	1.6	0.97	0.67
Mean	20	2.1	1.29	0.92

At present no obvious explanation can be offered in order to account for the increased concentration of unconjugated bilirubin in duodenal bile during phototherapy. From his rat experiments (9) Ostrow sets up the hypothesis that phototherapy brings about alterations in the bilirubin molecule or perhaps changes the permeability of the liver cell membrane in such a way that a free passive diffusion of bilirubin from blood to bile becomes possible. He supports this hypothesis by the fact that the concentration of unconjugated bilirubin in fistula bile during phototherapy at a maximum rose to a border line level at or just below the contemporary concentration of bilirubin in serum. In none of the patients of our study did the concentration of unconjugated bilirubin in duodenal bile exceed the concentration of bilirubin in serum. The maximum concentration of unconjugated bilirubin

was 75 mg/l The corresponding serum bilirubin concentration was between 218 and 170 mg/l If we suppose that liver bile in our patients by pancreatic and other secretions has been subjected to a two- or threefold dilution, which seems quite possible, we can bring no evidence against Ostrow s hypothesis On the other hand the possibility of a passive diffusion is not primarily determined by the *total* bilirubin concentration in serum and bile as bilirubin is bound firmly to albumin in serum and probably to phospholipids in bile (7)

The profound change in the colour of the duodenal bile observed during phototherapy cannot be explained by the alterations in the concentration of unconjugated bilirubin alone Conceivably this problem may be solved in connection with further identification of bilirubin photo oxidation products in the future

SUMMARY

The concentration of unconjugated bilirubin was measured in duodenal bile in 12 newborn infants with hyperbilirubinemia without signs of isoimmunization 7 infants received phototherapy for 24 hours between bile samples while 5 infants had no treatment and served as controls The concentration of unconjugated bilirubin in the duodenal bile was on the average doubled during phototherapy The findings are discussed in relation to similar studies performed in Gunn rats with bile fistula

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TOXIC EFFECTS IN THE GUNN RAT OF COMBINED TREATMENT WITH BILIRUBIN AND OROTIC ACID

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It is theoretically possible that lack of uridine coenzymes in the liver could be a factor limiting bilirubin conjugation in neonatal hyperbilirubinemia (4 9 12 16) Von Euler et al (11) have shown that the amount of uridine coenzymes in the liver of rats is increased by giving orotic acid a precursor in the biosynthesis of the uridine moiety It has accordingly been suggested (2 3) that orotic acid should be considered in the treatment of neonatal jaundice Kintzel et al (15) have found that total and indirect serum bilirubin levels in premature infants are decreased by treatment with orotic acid while no response was seen in full term newborns (19) These findings may very well be explained by a lack of UDPGA in the premature liver while the conjugating enzyme or other factors are limiting after term No toxic effects of orotic acid have been observed in several hundred children treated by Kintzel et al (15) but these authors call for a more careful examination of side effects It should be emphasized that a decreased level of plasma bilirubin is not a final criterion of therapeutic effect since the drug might cause a shift of bilirubin from blood plasma to the tissues (20) possibly including the brain It was accordingly decided to undertake a study of toxic effects and distribution of bilirubin after treatment with orotic acid. The Gunn (13) strain of rat was chosen as the experimental animal These rats are unable to conjugate bilirubin due to an inherited lack of the transferase Effects of orotic acid on the liver content of UDPGA therefore do not influence the distribution and toxicity of bilirubin thus allowing an isolated investigation of drug activities other than the effect on conjugation Results so far obtained are presented in the following

EXPERIMENTAL

^{14}C bilirubin was prepared from cat bile isolated and tested as previously described (5)

Gunn rats a mutant strain of Wistar (albino) rats with inherited absence of UDP glucuronate bilirubin glucuronyltransferase (13) were maintained by pure inbreeding through three generations after the parent animals had been obtained from Dr K P M Heirwegh Leuven Belgium The colony was kept at 22–24 C relative humidity 40 to 60 under fluorescent light 200 lux for 12 hours/day Neonatal mortality was high, occasionally with the majority of the young dying around the 5th day Skin and internal organs except the brain of the adult were markedly icteric In 7 animals the serum unconjugated bilirubin level ranged from 48 to 159 μM determined by the chloroform extraction method (7) Abnormal posture or gait was never observed in the untreated rats

Ordinary Wistar rats were used for comparative experiments The serum unconjugated bilirubin level in these was undetectable (below 0 5 μM)

Orotic acid was injected intraperitoneally (dose 600 mg/kg) given as sodium orotate dissolved in isotonic sodium hydroxide/sodium chloride at pH 10 0 6 mg orotic acid per ml injection fluid Control animals were given an equal volume of carbonate buffer/saline with the same pH and buffering capacity Due to the alkalinity of the injected solution the animals developed respiratory inhibition for about half a minute equally in orotate treated and controls

Table 1 *Toxic effects of orotate and bilirubin in Gunn rats*
Seven animals in each group Dose of orotate 600 mg/kg intraperitoneally Bilirubin 25 mg/kg intravenously

Group	0 hours	24 hours	30 hours	48 hours
1	Orotate 600 mg/kg pH = 10 0 i p	Saline pH = 10 0 i v	Slight hypothermia Normal postures and movements	Hypothermia Normal postures and movements
2	Saline pH = 10 0 i p	Bilirubin-Na 25 mg/kg pH = 10 0 i v	No signs	Hypothermia 2 rats Abnormal postures and movements
3	Orotate 600 mg/kg pH = 10 0 i p	Bilirubin-Na 25 mg/kg pH = 10 0 i v	Hypothermia 2 rats convulsions	All dead

Bilirubin was given intravenously about 24 hours after the orotate injection A single dose of 25 mg/kg was injected into a tail vein in 2 ml saline at pH 10 0 This amount is equal to 2 to 2 5 molecules of bilirubin per circulating molecule of plasma albumin 2 ml saline adjusted to pH 10 0 was given to control animals

Light ether anaesthesia was given before all injections

Rats were killed under ether anaesthesia by exsanguination from the heart Tissues for determination of ¹⁴C were removed homogenized and counted in a low level β counter as previously described (5) Corrections were made for background natural radioactivity of K and ¹⁴C and for self absorption, using a computer programme

Rectal temperatures were measured with an ordinary laboratory thermometer inserted to a depth of 2 5 cm

Displacement of bilirubin by orotate from binding to albumin was studied by recording light absorption spectra in a UNICAM SP 800 recording spectrophotometer and by the peroxidase method of Jacobsen (14) In this latter technique the very small concentration of free bilirubin anion in equilibrium with bilirubin bound to albumin is oxidized with hydrogen peroxide and horseradish peroxidase (6) Bound bilirubin is not oxidized The rate of oxidation is thus proportional to the concentration of free bilirubin

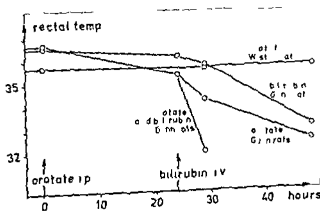


Fig 1 Rectal temperatures of orotate and bilirubin treated rats

RESULTS

Toxic effects of orotate and bilirubin

Five ordinary Wistar rats given orotate (600 mg/kg) showed no toxic signs and their body temperature remained normal

Gunn rats group 1

Seven Gunn rats were given orotate, 600 mg/kg Twenty four hours later the animals showed dark red urine In additional rats, similarly treated it was found that packed cell volume was markedly reduced at 24 hours and the blood plasma was bright red Body temperatures were normal at 24 hours and decreased to subnormal levels during the following day Otherwise the animals appeared normal at 48 hours when they were killed White deposits of unabsorbed orotic acid were seen in the abdomen In supplementary experiments some rats treated with this dose of orotate have died later than 48 hours (Table 1 Fig 1)

Gunn rats group 2

Seven Gunn rats were given no orotate but alkaline buffer/saline at the beginning of the

experiment and 24 hours later received an intravenous dose of bilirubin 25 mg/kg Signs of hemolysis were not noted at this dosage level but red urine was seen after 40 mg bilirubin per kg given to Wistar rats The bilirubin treated Gunn rats showed marked hypothermia 24 hours after the injection of bilirubin (48 hours after the start of the experiment as noted in Table 1 and Fig 1) Two rats showed abnormal postures and movements at this time It has been found in additional tests that some animals treated in this way die during the following days

Gunn rats group 3

Seven Gunn rats were injected with orotate and about 24 hours later with bilirubin dosages as above These rats developed a rapid drop of body temperature during 6 hours following the injection of bilirubin and 2 of the animals had convulsions at this time All rats in this group died within 24 hours of bilirubin treatment

Distribution of radioactive bilirubin in Gunn rats

Two groups each of 7 Gunn rats were treated as group 2) and 3) respectively with bilirubin alone and with orotate and bilirubin ^{14}C bilirubin 37 000 cpm/kg was included in the bilirubin dose The animals were killed after varying lengths of time and the ^{14}C content of blood plasma and blood cells was measured (Fig 2) ^{14}C bilirubin left the blood plasma faster in the animals pretreated with orotate than in the control group The radioactivity of the blood cells was not significantly different in the two groups

Radioactivities of liver kidneys small intestine and brain were measured No significant differences were found Brains were slightly radioactive approximately at a level corresponding to the expected radioactivity of trapped blood in the brain tissue Detailed studies of smaller sections of the brains are in

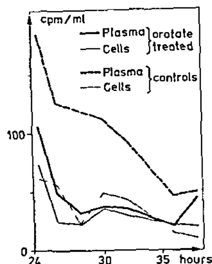


Fig 2 Radioactivity of plasma and blood cells of Gunn rats after intravenously administration of C bilirubin All animals were given bilirubin intravenously 25 mg/kg 37 000 cpm/kg Full lines Animals pretreated with orotate 600 mg/kg Dashed lines Control animals treated with bilirubin alone Heavy lines Blood plasma Light lines Blood cells

Displacement studies in vitro

Two methods were employed in order to examine whether orotate has a displacing effect on bilirubin bound to albumin Light absorption spectra of bilirubin–albumin complexes molar ratios 0 5 1 and 2 bilirubin per albumin were recorded without any displacing agent and with sodium orotate or sodium salicylate added to a concentration of 1 mmol/l No change of the spectrum was observed by addition of orotate irrespective of the bilirubin/albumin ratio Salicylate caused a shift of the spectrum towards shorter wavelengths with formation of a shoulder around 430 nm where the absorption maximum of free bilirubin is located This effect of salicylate was obvious at a molar ratio of 2 bilirubin to 1 albumin indicating displacement of bilirubin but was slight at the ratio 1 1 and hardly significant at 0 5 1 These findings show that orotate has no displacing effect on bilirubin at the 2nd and 3rd binding sites on the albumin molecule (These two sites have equal affinities and the second molecule of bilirubin is bound equally to both sites (14)) The concentration

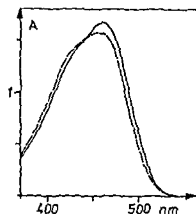


Fig 3 Light absorption spectra of bilirubin albumin 3 and 1 5 μmol/l respectively with 10 mmol/l NaCl 0 5 mmol/l EDTA 2 2 mmol/l formamide 10 μmol/l KCN pH 7 4 recorded in 10 cm cells Dashed line Sodium salicylate 1 mmol/l added Full line Control without salicylate

of free bilirubin at the ratio 2 1 is of the order of 0 5 μmol/l constituting a considerable fraction of the light absorbing pigment Any displacement which would cause a fair relative increase of free bilirubin would thus be seen from the spectrum At the molar ratio 0 5 1 of bilirubin to albumin however the concentration of free bilirubin is very low about 0 01 μmol/l Free bilirubin might increase 2- or 3 fold without causing a measurable change of the spectrum The spectrometric technique therefore is not suitable for observing displacement from the 1st site

The peroxidase method (14) is probably the only available technique for measuring free bilirubin concentrations of the order of 0 01 μmol/l and was employed for the study of displacement from the 1st site As seen in Table 2 no displacing effect of orotate could be demonstrated

Table 2 *The influence of orotate on the concentration of non bound bilirubin in a bilirubin–albumin solution, as measured by the velocity of oxidation with hydrogen peroxide and peroxidase*

Concentration of bilirubin and albumin in the reaction mixture both 44 μmol/l H_2O 840 μmol/l horseradish peroxidase 0 01 μmol/l

Orotate (μmol/l)	Oxidation velocity (ΔA_{555}/min)
0	0 045
238	0 056
476	0 045
952	0 049

DISCUSSION

As seen in Fig 2, an intravenous dose of radioactive bilirubin given to a Gunn rat leaves the blood stream much more rapidly in animals which have been pretreated with orotic acid, than in untreated controls A fairly constant level of radioactivity of the blood plasma is reached in about 2 hours in the treated animals and the activity remains constant for about 12 hours The level of radioactivity during this time is about 40 cpm per ml plasma The activity of the injected dose is 37 cpm/g body weight The distribution volume of the injected bilirubin is thus about 0 9 l/kg nearly equal to the total body volume This figure is in sharp contrast to the distribution volume in a normal human adult, 0 07 l/kg (1, 5)

The rapid disappearance of bilirubin from the blood stream in the treated animals could hardly be explained by an increased rate of bilirubin metabolism According to Schmid & Hammaker (18) the total miscible bilirubin pool of a Gunn rat is about 15 to 20 mg/kg with a turnover rate of 0 4 per 24 hours equivalent to metabolism of 0 6 mg bilirubin in 2 hours It seems extremely unlikely that the orotate treated animals would metabolize the major part of 25 mg/kg in 2 hours The effect of orotate is therefore to be sought in enhanced passage of bilirubin from the blood plasma into the tissues and altered distribution when equilibrium is reached

The altered distribution of bilirubin caused by orotate treatment is further illustrated by the ratio of ^{14}C bilirubin in plasma and red cells (Fig 2) In the untreated rats this ratio is about 2 4 plasma cells throughout the time of observation whereas the radioactivity is nearly equal in plasma and blood cells after orotate treatment

The rapid passage of bilirubin from blood

plasma to tissues and the altered distribution is apparently equivalent to a displacement of bilirubin from binding to serum albumin (8) As seen from the *in vitro*-experiments however no displacement takes place even at a high concentration of orotate 1 mmol/l The dose of orotate 600 mg/kg is equivalent to 4 mmol/l if equally distributed throughout the rat Since however a large amount of orotic acid remains at the injection site and since orotate is not normally present in blood plasma in appreciable amounts the plasma concentration 24 hours after the injection could maximally be 1 mmol/l and is probably much less

The effect of orotate *in vivo* accordingly must be on the membranes partly accelerating passage of bilirubin and partly making new areas in the organism accessible to bilirubin A toxic effect on membranes would also explain the intravascular hemolysis and hemoglobinuria observed in Gunn rats after treatment with orotate

This membrane toxic effect of orotate is seen in Gunn rats only not in the normal Wistar strain It is likely therefore that this effect is synergistic to the toxicity of bilirubin which affects membranes (10 17 21) Further evidence for the synergism of orotate and bilirubin in this respect may be obtained from the results pictured in Fig 1 and in Table 1 Hypothermia develops slowly after treatment with either substance and faster after the combined dosage Also the lethal effects are synergistic

It seems unlikely that toxic effects of orotate should be encountered in the treatment of newborns The Dresden group (15 19) has observed no toxicity The doses given 100 to 300 mg to a premature child approach the same order of magnitude as the dose given to the rats in the present study 600 mg/kg It is likely however that absorption from the gut is poor compared with the efficiency of i traperitoneal administration The synergistic membrane toxic effect of bilirubin and orotate may on the other hand prove of interest for th investigation of the toxic mechanism of bilirubin as well as for membrane biochemistry in a broader sense

SUMMARY

Intraperitoneal injection of sodium orotate (600 mg/kg) although non toxic to ordinary rats produced hemolysis and hypothermia in Gunn rats (icteric) Intravenous bilirubin (25 mg/kg) showed limited toxic effects When both substances were administered the animals rapidly became hypothermic and died within a few hours ^{14}C bilirubin given to Gunn rats left the blood plasma much more rapidly in animals pretreated with orotate than in untreated controls and showed a high distribution volume and binding to red cells *In vitro* no displacement of bilirubin by orotate from binding to albumin could be demonstrated The *in vivo* effect of orotate probably affects membranes potentiating the membrane toxic effect of bilirubin

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THE EFFECT OF OROTIC ACID ON THE BILIRUBIN ABSORPTIVE POWER OF PLASMA ALBUMIN IN NEWBORN INFANTS

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Orotic acid may be used as an aid to control toxic hyperbilirubinaemia in premature infants (as previously demonstrated in our department) (5 6) Administration of a daily dose of 300 mg of orotic acid led to a 90 % reduction in the number of replacement transfusions which normally become imperative at serum bili rubin levels above 18 mg per 100 ml Since then this effect has been observed in 245 prematurely born infants treated with orotic acid Orotic acid which is contained naturally in cow s milk (50–100 mg per litre) would thus lend itself to use as a prophylactic agent against bilirubin induced encephalopathy

However its large scale application cannot be recommended until it has been established that in plasma orotic acid is not coupled to albumin It was therefore the aim of the pres ent study to determine the degree to which the absorptive power of albumin is impaired by the action of orotic acid As no difference had been found between mature and prem ature infants in terms of intestinal absorp tion of orotic acid it was felt justified to conduct the investigation on infants born at full term as they constituted a substantially more homogeneous population with respect to plasma albumin concentrations In addition since the serum bilirubin levels of mature in fants were considerably lower only a minor proportion of the albumin s receptive capacity is bound by bilirubin Finally acidosis which is known to affect adversely the absorptive power of albumin need not be taken into consideration

METHODS

Preceding the clinical trial *in vitro* investigations were made to examine the effect of orotic acid on the absorptive power of plasma albumin Following de termination of the dyestuff binding capacity of al bumin from two serum specimens varying quantities of orotic acid ranging from 20 to 200 mg per 100 ml were added By the use of the Porter & Waters tech nique (7) which takes advantage of a shift in the absorption spectrum following addition of an azo dye [2 (4 hydroxyazobenzene) benzoic acid] to al bumin th absorptive power was measured photo metrically A solution containing 4 g albumin per 100 ml s rved as a blank

The *in vivo* investigations were made on 10 symp tomless full term infants receiving 200 mg of orotic acid per kilogram of body weight each from the first to the fifth day after birth. Another group of newborns equal in number served as a control On the first day after birth (i e prior to the medication period) the absorptive power of plasma albumin as well as serum bilirubin levels were determined from both groups with repeat measurements on the 3rd and 5th days in each case the absorptive power being measured according to the above mentioned procedure

RESULTS

The results of the in vitro experiments are summarized in Table 1 It is evident that in neither of the two sets of tests (first series 20–

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therefore not gain from orotic acid therapy whose mode of action is held to be chiefly substitutive Supposing orotic acid did have the property of displacing bilirubin from its albumin bond the daily administration to mature infants of 600 mg—a dose equivalent to the 300 mg given to premature infants—ought to have resulted in a lowering of serum bilirubin levels For the time being it may be stated that both the present results and those reported concurrently by Hinkel as well as by Splinter & Gmyrek all obtained by different techniques appear to invalidate the concept of bilirubin being expelled from its conjugation with albumin by orotic acid This is true also of earlier clinical investigations performed on full term infants A final judgement however will have to be deferred until the results of investigation being carried out by Brodersen (1) and a group working in close co operation with us have been concluded

SUMMARY

Prior to any large scale employment of orotic acid as a prophylactic aid for toxic hyperbilirubinaemia in premature infants evidence has to be accumulated as to whether this substance would cause bilirubin to disconjugate from albumin With the aid of a technique describ d by Porter & Waters the dyestuff absorptive power of plasma albumin has been examined *in vitro* and *in vivo* following addition or administration respectively of varying quantities of orotic acid The results obtained do not indicate that bilirubin may be disjoined from albumin by orotic acid These findings accord with earlier clinical investigations by the authors

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Table 1. *In vitro results*

First series						
0	20	40	60	80	100	mg of orotic acid per 100 ml of serum
123	123	125	123	123	125	absorptive power (°)
Second series						
0	40	80	120	160	200	mg of orotic acid per 100 ml of serum
131	130	133	130	133	131	absorptive power (°)

100 mg per 100 ml, second series 40–200 mg per 100 ml) did the absorptive power exhibit any appreciable change in relation to the initial values.

The results obtained from the *in vivo* experiments (Table 2) demonstrate that, in the control group, the absorptive power of albumin remained fairly constant as determined on the first, third and fifth day after birth. Infants receiving orotic acid exhibited values only negligibly lower than those of the control group with virtually no changes on the third and fifth day as compared with the original readings. The mean values of serum bilirubin levels were found to be too low to be likely to account for any major impairment of the absorptive power of albumin.

DISCUSSION

The in vitro investigations were conducted with concentrations of orotic acid ranging from very small quantities to doses unlikely

Table 2. *In vivo results*

	Absorptive power	Serum bilirubin (mg per 100 ml) Total	Indirectly reacting
Control group ($n = 10$)			
1st day	100.38 ± 3.99	4.28 ± 1.47	4.08 ± 1.32
3rd day	100.16 ± 11.1	8.02 ± 2.39	7.72 ± 2.05
5th day	99.17 ± 5.94	7.20 ± 2.89	7.06 ± 2.77
Orotic acid group ($n = 11$)			
1st day	96.98 ± 6.52	3.37 ± 1.47	3.26 ± 1.33
3rd day	97.83 ± 6.78	5.28 ± 2.22	5.13 ± 1.89
5th day	97.16 ± 3.52	3.65 ± 2.26	3.55 ± 2.24

ever to be reached in any scheme of treatment. The dosage of 200 mg per day per kilogram of body weight as administered during the *in vivo* experiments represents the maximum dose applied in current therapy. Addition or administration respectively of orotic acid has been shown to leave the absorptive power of plasma albumin unimpaired. With an eye to the possible clinical use of this substance in cases of hyperbilirubinaemia, it is essential to know whether bilirubin is displaced from its conjugation with albumin, the more so as orotic acid otherwise appears to be free from harmful side-effects if used in therapeutic doses (2, 4). To emphasize the urgency of investigating this problem by all means available, attention may be drawn to the ostensible nature of improvements attained by the use of Peristone (10).

With the Porter & Waters technique both *in vitro* and *in vivo* investigations failed to yield evidence of a displacement of the azo dye from its albumin bond by orotic acid. As bilirubin has a greater affinity to albumin than has the dyestuff used here, there is no reason to infer from the present results that bilirubin is forced out of its conjugation with albumin. This view has been corroborated by Hinkel (3) who, using the Sephadex gel filtration technique, also failed to demonstrate the existence of a combination between orotic acid and albumin. Likewise Splinter & Gmyrek (9), employing the dialysis technique according to Scholtan, arrived at similar results. Furthermore, clinical results obtained on full-term infants before these investigations were carried out did not indicate that bilirubin is displaced from its albumin bond by the action of orotic acid (8). By contrast with premature infants in whom it had a marked effect, full-term neonates when given 200 mg of orotic acid per day failed to show any reduction in serum bilirubin levels, with no improvement when the dosage was raised to 600 mg per day. It may be assumed that full-term infants have no deficiency in UDP, a coenzyme in the glucuronyl transferase system, and would

Table 1 *Mean weight and length of 27 infants followed from birth to 6 months of age*

Age (months)	Weight (kg)	Length (cm)
Birth	3 19	—
1	3 75	53 1
2	4 68	56.2
3	5.26	58.9
4	6 00	61 6
5	6 46	63 2
6	7 00	64 9

feedin of the infants were left entirely to the mothers except that breastfeeding was strongly recommended *Nearly half* of the infants were *from a low* socio-economic class with monthly income of less than US $40 and these infants were mainly breastfed but received fresh cow s milk supplements in rare in stances The rest were *from a higher* socio-economic group These infants were almost entirely fed com mercially available homogenized cow s milk prepara tions containing no additional iron with supplemen tary solid foods introduced after the age of 4 months The same proportion of the socio-economic groups was maintained up to 6 months of age All infants were apparently healthy and suffered only minor ill nesses such as mild diarrhoea and upper respiratory tract infections One infant developed pyogenic me ningitis at the age of 3 months and another contracted measles at the age of 5 months and these infants were excluded

RESULTS

All infants had satisfactory growth as shown in Table 1

The mean Hb PCV and mean corpuscular haemoglobin concentration (MCHC) values are shown in Table 2 For comparison the data obtained at sea level by Moe (8) from the city of Oslo Norway are inserted

The Hb and PCV values were very high at birth and subsequently fell progressively to reach the nadir at 3 months of age when the mean Hb and PCV were 10 9 g/100 ml and 38° respectively Thereafter there was a rise that was maintained throughout the remainder of the follow up period

DISCUSSION

The extremely high value for Hb at birth confirms the previous finding of Hofvander (6) and is probably due to late clamping of the cord combined with relative intra uterine hypoxia at high altitude In the study of Oh & Lind (10) the highest PCV was found in unwarmed heel samples

None of the infants in the present study were anaemic at birth that is with Hb less than 14 5 g/100 ml (11) This not surprising as Ethiopia is believed to be the country with the highest daily intake of dietary iron in the world and anaemia of pregnancy is virtually non existent (6)

The data obtained by us agreed very well with those of Moe (8) presumably at sea level up to 3 months of age However already at 4 months a highly significant increase ($p <$ 0 001) over the level at 3 months was demonstrated in our series from 10 9 to 12 5 g/100 ml and this level was maintained at 5 and 6 months of age These values are definitely

Table 2 *Haemoglobin and haematocrit values in the first 6 months*

		Present study altitude 2 400 m						Taken from Moe (ref 8) Sea level		
		Hb g/100 ml		PCV		MCHC				
Age (months)	No	M	S D	M	S D	M	S D	Hb g/100 ml	PCV	MCHC
Birth	14-	-3 0	2 8	74	7	30 9	1 9	19 8	66	30 0
1	74	13 3	0 9	44	6	30 1	2 6	14 1	45	31 1
2	57	11 5	1 3	39	1	29 5	3 0	11 4	38	30 1
3	5?	10 9	0 7	38	4	29 0	1 6	11 2	37	30 4
4	37	12.5	1 4	40	2	31 3	2 4	—	—	—
5	30	12.4	0 9	40	3	31 5	2 6	—	—	—
6	-7	12 6	0 8	39	2	32.8	2.3	11 5	38	30 0

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PHYSIOLOGIC ANAEMIA' OF INFANCY AT HIGH ALTITUDE

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Physiologic anaemia' of early infancy has been reviewed recently (9) It appears that at least three factors are involved in the development of postnatal decline of haemoglobin and haematocrit values decreased erythropoiesis, decreased red cell survival, and haemodilution as a result of rapid weight gain in the first few months of life Of these three factors decreased red cell production seems to be quantitatively the most important determinant contributing to the development of physiologic anaemia

It has been demonstrated that foetal red cell production is erythropoietin mediated and under conditions of hypoxic hypoxia and anaemic hypoxia high plasma erythropoietin levels have been recorded in cord blood (2 4) Postnatally commensurate with a rise in arterial oxygen saturation there is a sharp drop in the erythropoietin level (4) On the other hand, under conditions of hypoxia as in cyanotic congenital heart disease there is persistent elevation of erythropoietin (3) and postnatal decline in erythropoeisis is not observed (12)

Inverse correlation between haemoglobin level and percentage of arterial oxygen saturation resulting in high altitude polycythemia is well established (7) Erythropoietin is persistently elevated in subjects residing at high altitudes (1) An elevated level of erythropoietin is assumed to be produced in response to altitude hypoxaemia and accounts for the increased production of red cells Because postnatal decline in red cell production is due to lack of erythropoietin stimulation it is postulated that postnatal decline in haemoglobin (Hb) might be modified by altitude

To the best of our knowledge the effect of high altitude on the postnatal decline of Hb has not been investigated This communication reports on Hb and PCV values of apparently healthy infants living at high altitude and followed from birth to 6 months of age

MATERIAL AND METHODS

The study was conducted in Addis Ababa at an altitude of 2 400 m (8 000 feet) during the period December 1968 to May 1969 The material consisted of 142 consecutive normal single full term infants with a mean birth weight of 3 19±0 31 kg Eight infants (5 6) were delivered by Caesarean Section The rest were delivered vaginally and in these infants the cord was not clamped until all pulsations had ceased All infants were delivered at the Princess Tsehai Memorial Hospital and were examined by one of us before blood samples were obtained All infants were tested before 24 hours of age Prematures and sick newborns were excluded

Blood was obtained by finger prick unwarmed both in newborns and at follow up Hb and PCV values were determined in duplicate using the methods described previously (6) except that the heparinized capillary tubes were sealed with plastelina which sharply demarcates the lower end of the packed cell column

Nearly half of the original population was lost for follow up However once the infants were brought for routine checks at the age of 1 month the follow up rate was 77 at 2 months and 70 at 3 months Not all infants had reached the age of 4 months at the end of the study Twenty seven infants were followed to the age of 6 months

None of the infants had been treated with iron The

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RELATION BETWEEN CELLULAR IMMUNITY TO NEPHRO BLASTOMA AND THE PHASE OF THE DISEASE

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The correlation between the presence of tumour specific antibodies *in vitro* and the clinical tumour status has been examined in different tumour systems in man In a study of the humoral tumour related immunological reactions by means of the indirect fluorescent antibody assay Klein et al (4) observed the strongest reactivity with sera from patients with Burkitt s lymphoma in remission Likewise serum antibodies to tumour antigens were found in 80 % of a group of patients with malignant melanoma without disseminated disease They disappeared as the disease progressed (5)

Cell bound immunological reactions were examined by Chu et al (1) in patients with nasopharyngeal carcinomas These authors described a lower frequency of lymphocyte reactivity in tumour bearing subjects than in those without tumour In neuroblastoma patients a cell bound tumour related reactivity was found but no relationship with the stage of the disease was established (3) In studies by Chu et al (1) and by Hellstrom et al (3) the tumour bearing patients and those without tumours were not identical

The existence of cell bound tumour related immunity has been confirmed in autochthonous and allogeneic systems *in vitro* in a series of 24 patients with nephroblastoma (2)

The aim of this study was to investigate whether in individual patients with nephroblastoma changes in the specific tumour related immunity reflected in lymphocyte cytotoxicity *in vitro* have any correlation with the clinical course of the disease

MATERIALS AND METHODS

In 10 patients with nephroblastoma—4 boys and 6 girls aged 1–5 years on admission—the cytotoxicity of the lymphocytes for nephroblastoma cells was tested *in vitro* during the growth of the tumour and in periods of remission Each patient was tested 2–6 times and altogether 32 tests were performed

In all the patients surgical removal of the tumour had been followed by postoperative irradiation of the abdomen and administration of 60 gamma of actinomycin D per kilogramme of body weight on the first 6 postoperative days this drug therapy was repeated in 3 patients (WIL-1 WIL 11 WIL 16)

Irradiation in combination with actinomycin D and/or Oncovine was given for any metastases In 2 patients (WIL 1 WIL 16) pulmonary metastases were removed surgically Two patients (WIL 9 WIL-24) remained free of metasta es and in 2 patients (WIL 11 WIL-21) metastases appeared 3 years after the initial treatment One patient was given actinomycin D for 15 months as a prophylactic measure when pulmonary metastases were discovered 19 months after initial treatment In another metastases were found 6 months after initial treatment (WIL-16 WIL-17) In 4 patients pulmonary metastases were pre ent at the time of the first treatment (WIL-1 WIL-23 WIL-32 and WIL 33)

There were two control groups one consisting of 2? children hospitalized for non malignant surgical conditions and the other of 18 children with solid malignant tumours other than nephroblastoma and 4 healthy adults One person from each of these two groups was always taken as a control

On the day of the test peripheral blood counts of leukocyte and mononuclear cells were performed In

higher than those usually found at sea level (8) This suggests that the stimulus of high altitude for red cell production presumably through increased erythropoietin production starts to act sometime between 3 and 4 months of age

Our data indicate that high altitude corresponding to arterial oxygen saturation of 89% (5) has probably no appreciable effect on the development of postnatal decline in Hb during the first 3 months of life On the other hand erythropoietin inhibiting factor may be operative in postnatal decline in Hb (13) but the findings to date are tentative and await further confirmation Unfortunately we were unable to determine plasma and urinary erythropoietin levels in the infants we followed but such a study may shed more light on the mechanism underlying the development of the so called physiologic anaemia of the newborn

SUMMARY

The Hb and PCV values of apparently healthy infants living at an altitude of 2400 m was followed from birth to 6 months of age and the results were compared with a similar study at sea level

At birth the Hb and PCV levels were significantly higher in the present study than usually found at sea level The values found at 1 2 and 3 months, however were almost the same as those reported in infants at sea level By 4 months of age the mean Hb had increased to 12 5 g/100 ml, and this level was maintained almost unchanged at 5 and 6 months These values are again considerably higher than those usually found at sea level

This study suggests that high altitude (2400 m) has probably no appreciable effect on the development of postnatal decline in Hb and PCV It appears that the stimulus of high altitude for red cell production through increased erythropoietin production starts to act sometime between 3 and 4 months of age

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Table 1 *Lymphocyte cytotoxicity*

Expt. no	Nephro-blastoma patient no	Cells surviving after incubation (mean number of cells/well): Tumour tissue with lymphocytes from A Nephro-blastoma patient	B Control group	Normal tissue: C Nephro-blastoma patient	D Control group	Percentage specific cytotoxicity: on tumour tissue[b]	on normal tissue[c]	Level of significance of cytotoxic reduction of nephroblastoma p
7	1	23 3	34 3	39 8	39 0	32 1	−2.1	<0 005
23		7 2	25 8	—	—	72 1	—	<0 001
37		40 0	28 2	24 9	16 9	−41 8	−47 3	—
47		115 0	170 5	98 0	84 0	4 6	−16 7	—
10	7	31 3	37 8	51 0	56 0	4 6	8 9	—
16[d]		23 4	25 8	—	—	−10 1	—	—
34		6 6	16 2	50 1	87 2	59 3	42 6	<0 05
40		193 0	200 0	172 0	164 0	3 5	−4 9	—
50		40 7	56 5	12 6	38 1	28 0	66 9	—
2	11	16 9	15 7	45 1	33 7	−7 6	−33 8	—
45		48 0	33 5	95 0	110 6	−43 3	14 1	—
54[d]		135 0	152 0	45 0	42 0	11 2	−7 1	—
16[d]	16	14 8	25 8	—	—	42.6	—	<0 001
19		7 0	18 0	7 0	8 0	61 1	12 5	<0 001
29		11 6	25 0	7 0	8 0	53 6	12 5	<0 001
40		190 0	200 0	163 0	164 0	5 0	0 6	—
54[d]		179 0	152 0	39 0	42 0	−17 8	7 2	—
60		2 2	5 2	33 3	48 6	57 7	31 5	<0 05
34	19	17 3	16 2	83 4	87 2	−6 8	4 4	—
43		136 0	118 0	140 0	131 0	−15 3	−6 9	—
33	21	8 1	58 0	100 0	115 8	86 0	13 7	<0 001
38		68 0	60 0	17 5	16 5	−13 3	−6 1	—
40		207 0	200 0	168 0	164 0	−3 5	−2 4	—
51		64 0	38 5	—	—	−66 2	—	—
36	23	69 7	70 0	79 7	92 0	0 4	13 4	—
4		106 0	1 0 5	93 0	84 0	12 0	−10 7	—
34	24	15 9	16 2	79 4	87,2	1 9	9 0	—
45		7 0	33 5	81 0	110 6	79 1	26 8	<0 001
51[d]	32	11 0	38 5	—	—	71 4	—	<0 05
54		141 0	152 0	43 0	42 0	7 2	−2 4	—
51	33	79 0	38 5	—	—	24 7	—	—
54[d]		119 0	152 0	66 0	42 0	21 7	−57 1	<0 0125

[a] Calculated for a ratio target/effector cells of 1 250–500 6–20 wells counted in each group
[b] 100−(A/B 100) [c] 100−(C/D 100)
[d] High percentage of granulocyte contamination

The results are summarized in Table 3 In only one out of 15 tests in the patients with active disease did lymphocytes react whereas in 10 out of 17 tests in patients in remission the reactivity was higher than that of control lymphocytes

The relationship between the cytotoxic reaction pattern and the clinical course and therapy is represented graphically in Figs 1–3 Patient WIL 1 (Fig 1) developed lung metastases on three occasions in 14 months During remission a fairly strong specific cellular reactivity was recorded but decreased when treatment was given for secondary metastases

Patient WIL 7 (Fig 2) recorded negative tests 2 weeks before clinical diagnosis of secondary lung metastases remained unreactive during treatment and developed cellular cytotoxicity during remission Two months later when a new relapse was diagnosed the lymphocytes did not destroy tumour cells *in* [illegible] fter successful treatment

some of the patients a slight suppression of one or both counts was observed during and after treatment

The test consisted in measuring whether the lymphocytes of nephroblastoma patients showed *in vitro* higher cytotoxicity for nephroblastoma cells than for non malignant cells It was performed by adding the lymphocytes of the nephroblastoma patients and of controls to the target tissue which consisted of tissue cultured cells derived through different passages from nephroblastoma respectively normal cells

The target tumour tissue was obtained from 3 primary tumour patients (WIL T 5 WIL T 23 WIL T 25) and 1 patient (WIL T 1) who had had pulmonary metastases removed on two occasions The surrounding pulmonary or renal tissue in the surgical specimen served as normal control target tissue The diagnosis of the tumour and the normal target tissue was verified by histological examination in all cases The tumour of case WIL T 5 was the most viable tumour *in vitro* and served as tumour target tissue in 20 tests

The surgical specimens were sampled for tissue culture in a sterile medium (BME) Dispersed tissue was trypsinized and the cells were cultured in 250 ml square glass bottles and glass Petri dishes

The separation of mononuclear cells from polynuclear cells was effected by incubation of 10-20 ml of heparinized peripheral blood with iron powder or in a 50 ml nylon fibre column The mean granulocyte count was 22 ranging from zero to 60 The difference in the percentage granulocyte contamination in the control and nephroblastoma patients (compared in each experiment) was 0 to 10% in 24 experiments and 11 to 35 in 8

Target cell destruction as a measure of the cytotoxic activity of the lymphocytes was investigated in Falcon plastic dishes by a procedure described by Takasugi & Klein (6) About 100-300 target cells per well were seeded in approximately 10 μl of growth medium Plates were incubated for 48 hours the cells were re fed after 24 hours and debris and unattached intact cells were removed

Effector cells were added in target to effector cells ratios 500 1 or 250 1 Incubation of the test plates was carried out for 3-5 days then the lymphocytes were washed off After fixation and staining the remaining target cells were counted

The result of the test was expressed as the percentage reduction of the target cells by the effector cells It was taken as positive when the reduction recorded for a nephroblastoma patient was significantly greater than that for the control groups The level of significance was calculated by Student s t test

A detailed description of the cytotoxicity test has been given earlier (2)

RESULTS

The numbers of tumour and normal cells surviving incubation with test and control lymphocytes are shown in Table 1, together with the levels of significance Twenty-one out of 32 tests showed positive values but in only 11 was the difference between the test and control groups significant The reaction was usually less cytotoxic on normal than on tumour tissue

The mean reduction values for the various tumour target cells were between 22 3% and 35 8% It was 29 6% for WIL T 5 There was no significant difference in the reactivity of the various tumour tissues to cytotoxic lymphocytes from the investigated individuals (2)

The treatment the clinical stage at the time of the test and the results are presented in Table 2 In general a positive test result was related to the absence of clinical manifestations of tumour An exception was a nephroblastoma patient (WIL 19) who was free of symptoms for more than a year and displayed no signs of cellular immunity to nephroblastoma tumour cells *in vitro* In another patient (WIL-11) metastases were detected after 40 months and in the course of radiotherapy remission ensued without the development of lymphocyte reactivity

The opposite reaction—active disease simultaneous with cellular reactivity—was observed in 1 patient (WIL 16) after 3 months of treatment

The immunological reactivity was not suppressed by chemo- or radiotherapy alone (WIL 16 and WIL 24)

The results were both positive and negative when lymphocytes from the same nephroblastoma patients were added to the tumour target tissue from the same donor in different tests (patients WIL T 16 WIL T 32 WIL T 33) On the other hand when lymphocytes from one patient were added to tumour target cells from two different donors in the same experiment the results were either positive or negative in both tests (patients WIL-16 WIL 19 WIL 23)

In tissue cultures from four different donors the results were neither consistently positive nor consistently negative

Tabl 1 *Lymphocyte cytotoxicity*

Nephro-blastoma patient no	Cells surviving after incubation (mean number of cells/well)[a] Tumour tissue with lymphocytes from: A Nephro-blastoma patient	B Control group	Normal tissue: C Nephro-blastoma patient	D Control group	Percentage specific cytotoxicity on tumour tissue[b]	on normal tissue[c]	Level of significance of cytotoxic reduction of nephroblastoma p
1	23 3	34 3	39 8	39 0	32 1	−2 1	<0 005
	7 2	25 8	—	—	72 1	—	<0 001
	40 0	28 2	24 9	16 9	−41 8	−47 3	—
	115 0	170 5	98 0	84 0	4 6	−16 7	—
7	31 3	37 8	51 0	56 0	4 6	8 9	—
	28 4	25 8	—	—	−10 1	—	—
	6 6	16 2	50 1	87 2	59 3	47 6	<0 05
	193 0	200 0	172 0	164 0	3 5	−4 9	—
	40 7	56 5	12 6	38 1	28 0	66 9	—
11	16 9	15 7	45 1	33 7	−7 6	−33 8	—
	48 0	33 5	95 0	110 6	−43 3	14 1	—
	135 0	152 0	45 0	42.0	11 2	−7 1	—
16	14 8	25 8	—	—	42 6	—	<0 001
	7 0	18 0	7 0	8 0	61 1	12 5	<0 001
	11 6	25 0	7 0	8 0	53 6	12 5	<0 001
	190 0	200 0	163 0	164 0	5 0	0 6	—
	179 0	152 0	39 0	42 0	−17 8	7 2	—
	22	5 2	33 3	48 6	57 7	31 5	<0 05
19	17 3	16 2	83 4	87 2	6 8	4 4	—
	136 0	118 0	140 0	131 0	−15 3	−6 9	—
21	8 1	58 0	100 0	115 8	86 0	13 7	<0 001
	68 0	60 0	17 5	16 5	−13 3	−6 1	—
	207 0	200 0	168 0	164 0	−3 5	−2 4	—
	64 0	38 5	—	—	−66.2	—	—
23	69 7	70 0	79 7	92 0	0 4	13 4	—
	106 0	170 5	93 0	84 0	12 0	−10 7	—
24	15 9	16 2	79 4	87 2	1 9	9 0	—
	7 0	33 5	81 0	110 6	79 1	26 8	<0 001
32	11 0	38 5	—	—	71 4	—	<0 05
	141 0	152 0	43 0	42 0	7 2	−2 4	—
33	79 0	38 5	—	—	24 7	—	—
	119 0	152 0	66 0	47 0	21 7	−57 1	<0 0125

[a] Calculated for a ratio target/effector cells of 1 250–500 6–20 wells counted in each group
[b] 100 (A/B 100) [c] 100−(C/D 100)
[d] High percentage of granulocyte contamination

The results are summarized in Table 3 In only one out of 15 tests in the patients with active disease did lymphocytes react whereas in 10 out of 17 tests in patients in remission the reactivity was higher than that of control lymphocytes

The relationship between the cytotoxic reaction pattern and the clinical course and therapy is represented graphically in Figs 1–3 Patient WIL 1 (Fig 1) developed lung metastases on three occasions in 14 months During remission a fairly strong specific cellular reactivity was recorded but decreased when treatment was given for secondary metastases

Patient WIL-7 (Fig 2) recorded negative tests 2 weeks before clinical diagnosis of secondary lung metastases remained unreactive during treatment and developed cellular cytotoxicity during remission Two months later when a new relapse was diagnosed the lymphocytes did not destroy tumour cells *in vitro* but did so after successful treatment

Table 2 *Lymphocyte reaction in relation to treatment and phase of nephroblastoma*

Donor of targer cells		Nephro blastoma pat no	Date of test	Treatment and phase of the disease at the time of testing		Reaction
WIL-T-1		1	3 11 69	–	Remission	+
1			12 2 70	–	Remission	+
23			20 4 70	Irrad + Oncovine	Active	–
5			2 6 70	–	Remission	–
WIL-T-1		7	18 11 69	Irrad + AMD[a]	Active	–
1			9 1 70	Irrad + Oncovine	Active	–
25			20 3 70	–	Remission	+
5			26 5 70	Irrad + Oncovine	Active	–
5			13 7 70	–	Remission	–
WIL-T-1		11	5 2 70	Irradiation	Active	–
5			12 6 70	–	Remission	–
5			27 10 70	–	Remission	–
WIL-T-1	WIL-T-23	16	9 12 69	AMD	Remission	+
1	23		26 2 70	AMD	Remission	+
5			26 5 70	–	Remission	+
5			27 10 70	Irrad + Oncovine	Active	–
5			26 11 70	Oncovine	Active	–
5			21 1 71	Oncovine	Active	+
WIL-T-25		19	19 3 70	–	Remission	–
5			5 6 70	–	Remission	–
WIL-T-25		21	13 3 70	–	Remission	+
5			11 5 70	Irrad + Oncovine	Active	–
5			26 5 70	Oncovine	Active	–
5			29 9 70	–	Active	–
WIL-T-1	WIL-T-5	23	16 4 70	AMD + irrad	Active	–
5			2 6 70	–	Active	–
WIL-T-25		24	19 3 70	AMD + irrad	Remission	–
5			12 6 70	–	Remission	+
WIL-T-5		32	22 9 70	–	Remission	+
5			27 10 70	Irrad + AMD + Oncovine	Active	–
WIL-T-5		33	29 9 70	Irrad + AMD + Oncovine	Active	–
5			27 10 70	AMD + Oncovine	Remission	+

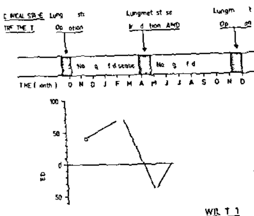
[a] Actinomycin D

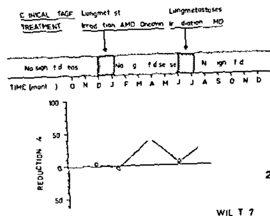
Table 3 *Cytotoxic reaction in relation to the phase of nephroblastoma and treatment*

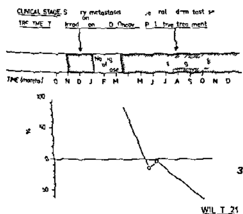
	Cytotoxic tests		
	Positive	Total	Percentage positive
Active disease			
Treatment	1	13	8
No treatment	0	2	—
Total	1	15	7
Disease in remission			
Treatment	3	4	75
No treatment	7	13	54
Total	10	17	59

In patient WIL 21 (Fig 3) the first metastases appeared in the skeleton 3 years after the initial treatment As this was an unusual course for nephroblastoma histological confirmation of the diagnosis was obtained from several pathologists In spite of a high *in vitro* immunological reaction in March generalized disease developed 2 weeks later and no lymphocyte reactivity was observed

There is no relationship between the results of the test and the leukocyte and mononuclear cell counts The lowest such counts in the patient with nephroblastoma recording a posi







Figs 1–3 Graphic demonstration of the percentage cytotoxic reduction in three nephroblastoma patients in correlation to clinical stage and treatment

the test were 1 900/mm³ and 380/mm³ respectively with corresponding means of 4 700/mm³ and 1 400/mm³ The respective means in the cases where the lymphocyte cytotoxicity test was negative were 4 400/mm³ for leukocytes and 1 500/mm³ for mononuclear cells

DISCUSSION

The present findings confirm earlier observations (2) that tumour related cell mediated cytotoxicity *in vitro* occurs less frequently in nephroblastoma patients with disseminated disease than in those without metastases The results suggest a correlation between lymphocyte cytotoxicity against tumour-cells and the clinical course of the disease Lymphocytes from patients with active disease were reactive only in 7 % compared with 59 % in patients in clinical remission

Treatment inducing remission did not seem to influence the reactivity to the same degree In spite of peripheral lymphopenia with the lowest value of 380 mononuclear cells per mm³ positive cytotoxicity in tumour free patients was observed in 75 % during and in 54 % without treatment

Considerable difficulties associated with a study of this type incur potential sources of error Firstly satisfactory planning of the tests is complicated by the small number of patients and their erratic availability Moreover the viability of the tumours in the tissue culture is highly unpredictable Secondly the case series is selected patients with metastases predominating a circumstance that might partly account for the fairly high frequency of negative tests Thirdly the outcome of the cytotoxicity test may be influenced by the concomitant immuno-depressive radio- or chemo

Table 2 *Lymphocyte reaction in relation to treatment and phase of nephroblastoma*

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5		13 7 70	–	Remission	–
WIL-T-1	11	5 2 70	Irradiation	Active	–
5		12 6 70	–	Remission	–
5		27 10 70	–	Remission	–
WIL-T-1 WIL-T-23	16	9 12 69	AMD	Remission	+
1 23		26 2 70	AMD	Remission	+
5		26 5 70	–	Remission	+
5		27 10 70	Irrad + Oncovine	Active	–
5		26 11 70	Oncovine	Active	–
5		21 1 71	Oncovine	Active	+
WIL-T-25	19	19 3 70	–	Remission	–
5		5 6 70	–	Remission	–
WIL-T-25	21	13 3 70	–	Remission	+
5		11 5 70	Irrad + Oncovine	Active	–
5		26 5 70	Oncovine	Active	–
5		29 9 70	–	Active	–
WIL-T-1 WIL-T-5	23	16 4 70	AMD + irrad	Active	–
5		2 6 70	–	Active	–
WIL-T-25	24	19 3 70	AMD + irrad	Remission	–
5		12 6 70	–	Remission	+
WIL-T-5	32	22 9 70	–	Remission	+
5		27 10 70	Irrad + AMD + Oncovine	Active	–
WIL-T-5	33	29 9 70	Irrad + AMD + Oncovine	Active	–
5		27 10 70	AMD + Oncovine	Remission	+

[a] Actinomycin D

Table 3 *Cytotoxic reaction in relation to the phase of nephroblastoma and treatment*

	Cytotoxic tests		
	Positive	Total	Percentage positive
Active disease			
Treatment	1	13	8
No treatment	0	2	–
Total	1	15	7
Disease in remission			
Treatment	3	4	75
No treatment	7	13	54
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There is no relationship between the results of the test and the leukocyte and mononuclear cell counts The lowest such counts in the patient with nephroblastoma r

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LACTOSE INTOLERANCE IN NIGERIAN CHILDREN

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Previous reports have shown that lactose in tolerance due to intestinal lactase deficiency in apparently healthy adults is widely pre valent in the non Caucasian races (1 2 4 8 9 11 13 18 19 22–24 28 31 32 35) and in Greek Cypriots (27) The aetiology of the deficiency is still controversial Most authors favour a genetic deficiency in lactase produc tion which may be peculiar to the peoples who are lactose intolerant (2 3 8–10 14 16–19 2? 24 27 29 36) Others suggest a gradual adaptive decline in enzyme activity due to lack of continued substrate challenge in the form of low milk consumption after weaning (4 5 11 13 28)

In a recent paper it was reported that lac tase deficiency was prevalent among adults belonging to all the ethnic groups of Nigerians resident in Ibadan Among the Yoruba the main ethnic group lactose intol rance was found in 84 of the 48 subjects investigated (30)

In the present study the pattern of lactose intolerance in Yoruba children was investi gated to determine its age of onset and rela tionship with the low milk intake after weaning Such a study might help to clarify whether in this society where breast feeding is universal but milk intake after weaning is low the high prevalence of lactase deficiency in adults can be explained on a genetic or an adaptive basis

Lactose and milk tolerance tests were also performed on a group of children presenting with protein calorie malnutrition (kwashi orkor)

MATERIALS AND METHODS

Selection of patients

The 89 Nigerian children investigated were born of unrelated Yoruba parents resident in Ibadan

The subjects were divided into three groups The first comprising 28 unweaned children (16 males and 12 females) was randomly selected from healthy and apparently well nourished children attending the In fant Welfare Clinic of the Ibadan City Council Maternity Centre Inalende Ibadan All the children were born at the Centre The average age was 6 4 months (range 1 to 14 months) and the average weight was 6 5 kg (range 2 8 to 10 kg)

The second group of 38 weaned children (25 fe males and 13 males) was randomly selected from children attending the General Outpatient s Clinic of University College Hospital for the first time The average age was 5 3 years with a range of 2 to 12 years The average age at weaning was 20 months (range 8 to 30 months) and the average weight was 15 3 kg (range 8 to 34 kg)

None of the children was suffering from diabetes or di ease of th gastro intestinal system All of them appeared well nourished. The main diagnoses were malaria and upper respiratory tract infections

The third group comprising 23 children (11 males and 12 females) presenting with protein calorie mal nutrition was also selected from the General Out patient s Clinic of University College Hospital Diag nosis was established by the presence of peripheral oedema mental apathy characteristic hair and skin changes and low total serum proteins and serum albumin The average age was 2 /₃ years (range 2 to 6 years) and the average weight was 8 kg (range 5 3–11.2 kg)

therapy However, further evidence that the results of the test are correlated with the course of the disease rather than with the treatment is provided by the following observations In one patient with progressive disease who was not treated (WIL-21) the test was negative while another patient in remission (WIL-16) who was treated with actinomycin D for 15 months recorded 3 positive tests during this time when the treatment was discontinued and metastases appeared the test was negative

SUMMARY

The correlation between cell mediated tumour related cytotoxicity and the clinical course of the disease has been examined by a cytotoxic microtechnique in 10 patients with nephroblastoma There was a relationship between the reactivity of the lymphocytes and the activity of the disease In only one out of 15 tests did lymphocytes from patients with clinically manifest tumours show a positive reaction compared with 10 out of 17 tests in patients in remission

ACKNOWLEDGEMENT

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dren (79°) but none of the 28 unweaned children were lactase deficient Of the 23 children with protein calorie malnutrition 7 (30%) were lactose intolerant and 6 of these also failed to show a rise in blood sugar great r than 20 mg per 100 ml after milk ingestion

The results of the glucose galactose tolerance tests in the weaned children showed that 5 of them also had a maximum rise in blood sugar b low 20 mg per 100 ml (Fig 1) The average rise in blood sugar for the group was 35 mg per 100 ml (range 8 to 75 mg/100 ml) following the administration of glucose galactose Control studies with glucose galactose were not performed on the unweaned children because all of them had normal tolerance to lactose Normal tolerance to glucose and galactose was recorded in the malnourished children who were intolerant to lactose and milk

Symptoms of diarrhoea following lactose ingestion were recorded in 11 of the weaned children but in none of the unweaned group In the kwashiorkor group diarrhoea was reco ded in all the children intolerant to lactose and milk Diarrhoea usually started within 2 hours of lactose or milk ingestion and stopped within 12 hours The numbers of stools ranged from three to seven Owing to the outpatient nature of the study the pH of the stools could not be determined In addition to the diarrhoea increased abdominal noises were reported in some of the younger children and a few of the older ones also complained of abdominal discomfort

DISCUSSION

It has previously been reported that lactase deficiency is common in apparently healthy Yoruba adults aged from 13 to 70 years (30) The results of the present study indicate that adequate amounts of intestinal lactase are p esent in Yoruba children during breast feeding The levels of lactase seem to fall off after weaning when carbohydrates and starches constitute the main food items All the 28 unweaned children (100°) but only 8 out of 38 (21%) of the weaned children were lactose tolerant The results thus demonstrate that lactase activity is related to breast feeding in Yoruba children A similar post weaning decline in lactase activity has been reported in Baganda children (10) in Singapore (5) and in Thailand (23)

The infant feeding practices of Yoruba mothers have been described previously In summary lactation is adequate and breast feeding universally practised (26) Breast feeding is commenced within 24 hours of child birth and continued until the next pregnancy when the child is completely weaned onto adult foods (21) (25) Deficiencies in lactation are related to low yields rather than to a qualitative defect in the breast milk (20) Supplementary foods are mainly carbohydrates and starches *Eko mimu* (maize gruel) boiled yams bread boiled rice and *gari* (a cassava preparation) These are gradually introduced as from the age of 4 to 6 months and weaning is completed by 36 months (21) A few mothers depending on their financial means supplement their children s diet with imported dried milk and commercial baby foods but as from the time of complete weaning and through adult life milk and milk products do not constitute important items of the Yoruba diet The national average daily milk consumption in Nigeria has been estimated at 32 grams (33) and the Yorubas are not likely to exceed this average However as an increasing number of children now drink milk after weaning it would be of interest to find out whether the onset of lactose intolerance would be delayed in them Such long term studies are in progress

Although the data presented in this paper do not permit firm conclusions on whether the aetiology of adult lactase deficiency in this environment can be explained on a genetic or an adaptive basis they appear to us to be more compatible with the latter We have found that the prevalence of adult lactase deficiency in other unrelated ethnic groups of Nigerians

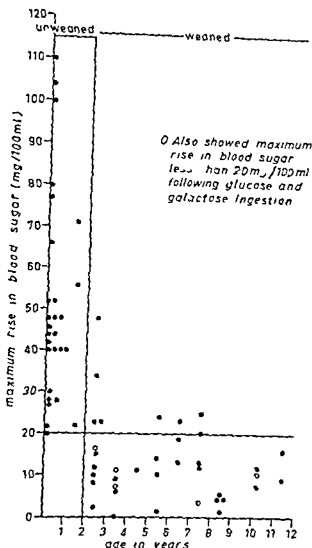


Fig 1 Lactose and glucose galactose tolerance tests

Tolerance tests

All investigations were carried out at the Metabolic Research Unit of University College Hospital Ibadan The tests were performed after an overnight fast on the weaned and kwashiorkor children and after a 6 hour fast on the unweaned children

Lactose tolerance tests with 2 g lactose/kg body weight for children weighing under 25 kg or 1 g/kg body weight for those weighing more were performed first The lactose was administered as a 10% solution which was ingested within 5 min Capillary blood samples were taken at 0 15 30 60 and 90 min by heel or finger prick and the levels of total blood reducing substances (blood sugar) were determined by a modification of the method of Folin & Wu (34) To test for glucose galactose intolerance the same procedures were repeated 24 or 48 hours later on the weaned children after the administration of 1 g each of glucose and galactose per kg body weight Any symptoms produced after the lactose or glucose galactose drink were noted and the mothers were questioned about the number of bowel motions after the lactose or glucose galactose drink

For the milk tolerance tests a commercial product containing 53 4 g lactose per 100 g milk powder (SIMILAC Abbott Laboratories Ltd Chicago Ill USA) was administered in sufficient quantity to ensure a lactose content of 2 g/kg body weight per test dose The amount of water added to the milk powder was similar to that recommended for a single feed The symptoms reported after milk ingestion were also noted

RESULTS

The individual results of the tolerance tests are shown in Figs 1 and 2 Following the administration of lactose the average maximum rise in blood sugar above fasting levels in the unweaned group was 51 mg per 100 ml (range 20 to 110 mg/100 ml) and 8 mg per 100 ml (range 0 to 48 mg/100 ml) in the weaned children (Fig 1) In the kwashiorkor group the average rise in blood sugar was 30 mg per 100 ml (range 4 to 63 mg/100 ml) after lactose and 24 mg per 100 ml (range 2 to 47 mg/100 ml) after milk ingestion (Fig 2)

If a maximum rise in blood sugar level below 20 mg per 100 ml after the ingestion of lactose is considered indicative of lactase deficiency (15) 30 out of the 38 weaned chil

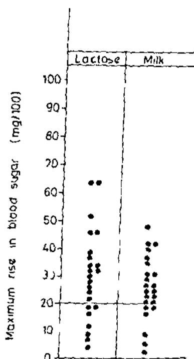


Fig 2 Lactose and milk tolerance tests in protein calorie malnutrition

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Key words Nigerian children lactose and milk intolerance

living in the Southern parts of the country, where pastoralisation and dairying are also virtually unknown approaches 100% Milk as a food item for adults is new to this environment and, indeed, there is no Yoruba word for milk There is also no evidence that the historical cross cultural contacts between the peoples of the Middle East where dairying originated and spread to Western Europe, and the Yoruba influenced the dietary habits of the latter One could therefore conclude that the Yoruba people have not avoided dairying because they are primarily lactase deficient They also do not rear cattle mainly because the vegetation in their environment has made this impossible The falling off in intestinal lactase levels after weaning can be explained by a lack of substrate challenge resulting from low milk intake

Lactose intolerance was specific in 33 of the 38 weaned children Secondary causes may have been responsible for the intolerance in the 5 children who also failed to show a rise in blood sugar greater than 20 mg per 100 ml following the ingestion of glucose and galactose

The close relationship between the age of weaning when protein calorie malnutrition normally commences and the onset of lactose intolerance as shown in this study raises some practical problems about the use of milk in the treatment of malnutrition Attention has been drawn in the past to lactose intolerance as an important factor in the pathogenesis of diarrhoea of protein calorie malnutrition (6 7 12 37) The ingestion of milk with a lactose content equal to that employed in lactose tolerance tests has been shown to produce symptoms Whilst it is possible that a smaller test dose of milk might produce fewer symptoms there is need for some caution in the use of milk in the treatment of protein calorie malnutrition in an environment where the majority of the weaned children are lactose intolerant The post weaning decline in lactase activity in these children points to a need for increasing use of lactose free diets and milk substitutes in supplementation feeding of infants and in the treatment of protein calorie malnutrition

SUMMARY

Lactose tolerance tests were performed on 89 Nigerian children born of unrelated Yoruba parents resident in Ibadan Intolerance to lactose was present in 30 out of 38 weaned children aged 2 to 12 years (79%) but in none of 28 unweaned children aged from 1 month to 14 months Out of 23 children suffering from protein calorie malnutrition (kwashiorkor) 7 (30%) were lactose intolerant and 6 of these were also intolerant to milk Lactose intolerance started shortly after weaning and the prevalence from then on in the age group 2 to 12 years (79%) was similar to that previously reported in the adult Yoruba population

Adult lactase deficiency in this population is explained as a failure in adaptation resulting from the low milk intake after weaning The widespread occurrence of lactose intolerance in this population and the production of symptoms following milk ingestion in children with kwashiorkor suggest that some caution is necessary in the large scale use of milk for protein replenishment in this environment

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SHORT COMMUNICATION

THE ELIMINATION OF ALCOHOL IN THE PREMATURE INFANT

U GÄRTNER and G RYDÉN

From the Department of Paediatrics and the Department of Obstetrics and Gynaecology University Hospital Linköping Sweden

Since Fuchs et al (2) published their results concerning the inhibitory effect of ethanol on premature labour, this kind of treatment in threatened premature delivery has been an accepted procedure in many clinics Little information is available, however, concerning the effect of ethanol on the premature infant and if the treatment of the mother is unsuccessful, the infant may be born with a rather high blood concentration of ethanol, which might have an adverse effect on the child

MATERIAL AND METHODS

The present investigation is limited to a study concerning the elimination rate of ethanol in three premature and one small for date infant where the treatment with ethanol for premature labour was unsuccessful

Ethanol was given intravenously according to Fuchs et al (2)

Capillary blood samples were taken from the newborn infant at regular intervals during 4 to 14 hours In two cases venous blood samples were taken from the mother and in three cases from the umbilical cord after delivery

The amount of ethanol in the blood was estimated using gas chromatography Blood sugar was estimated using the glucose oxidase method

RESULTS

The results obtained are summarized in Table 1 The elimination of ethanol in blood was found to follow a zero order reaction The disappearance rate of ethanol from the blood of the 4 infants was 8 9, 12 and 20 mg per 100 ml and hour, respectively

No clinical signs of hypoglycemia were observed Blood sugar estimations were taken during the first 3 or 4 days of life and were normal i e more than 20 mg per 100 ml

One of the infants died on the 4th day of life from respiratory insufficiency At autopsy immature lungs were found on microscopical examination

Patients 1 and 4 showed no signs of respiratory insufficiency had a normal weight gain and were normal at neurological examination at 5 and 9 months of age Patient 3 was delivered by Cesarean section due to vaginal atresia This patient with the highest rate of ethanol elimination, showed the first signs of respiratory insufficiency after about 4 hours of life Normal respiratory function was obtained on the 4th day of life

DISCUSSION AND CONCLUSION

Pikkarainen & Raiha (3) have studied the occurrence of alcohol dehydrogenase (ADH) and its isoenzymes on the human foetal early postnatal infant and adult liver They concluded that adult values are not reached until 5 years of age Wagner et al (5) studied the elimination of ethanol after intravenous infusion in the umbilical vein in six low birth weights infants (2 000 to 2 500 g) They calculated the elimination rate of ethanol to be

Table 1 *Summary of clinical findings*

Patient	Ethanol in blood at delivery (mg/100 ml) Maternal	Umbilical vein	Gestational age	Apgar score after 1 min	Weight (g)	Length (cm)	Rate of ethanol elimination (mg/100 ml blood and hour)
1	60	72	34	8	2 150	45	12
2	—	—	30	6	1 740	43	8
3	87	102	37?	8	1 630	40	20
4	—	183	33	1 min 3 5 min 6	1 130	37	9

Patient 2 died on the 4th day in respiratory insufficiency Microscopical examination showed immature lungs
The gestational age of patient 3 was uncertain After postnatal examination the gestational age was calculated as 37 weeks

7 4 mg per 100 ml blood and hour on average while a small for date infant eliminated ethanol at almost the adult rate In the present study where the three infants weighed less than 2 000 grams the rate of ethanol elimination was 8 9 12 and 20 mg per 100 ml blood and hour respectively This can be compared to the average rate in the adult 15 mg (1) The present results thus indicate that highly immature infants also have enzyme systems capable of metabolizing ethanol The elimination rate seems however to be somewhat slower in the immature infant than in the adult This is also indicated by the fact that the concentration of ethanol at delivery is lower in the maternal blood than in the umbilical cord blood The same observation was made in the single case reported by Fuchs et al (2) and Seppala et al (4)

We have not been able to demonstrate any significant depression on the central nervous system in the infants studied Further studies are indicated however to establish the risks of alcohol treatment in premature labour Until such information concerning the effect of ethanol on the premature infant is available this kind of treatment should be used with caution

SUMMARY

In 3 premature and one small for date infant which were born despite alcohol treatment of their mothers because of premature labour the rate of elimination of ethanol in the blood has been followed The elimination rate was fastest 20 mg per 100 ml and hour in the infant with the longest gestational age —37 weeks—compared with 8 9 and 12 mg in the infants with 30 33 and 34 weeks of gestation respectively The results indicate that highly immature infants are capable of metabolizing ethanol The ethanol elimination rate is however slower in the immature infant than in the adult

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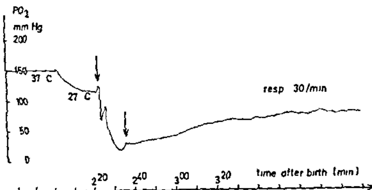


Fig 2 Oxygen tension during umbilical artery catheterization The introduction of the catheter was begun at the first and completed at the second arrow

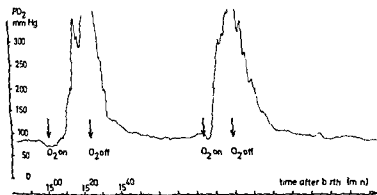


Fig 3 Increase in arterial oxygen tension after 75 oxygen administration to the infant at 15 minutes after birth

indicates that in the case illustrated the total shunting was < 10

Our pilot study has shown the efficacy of this catheter oxygen electrode and we will continue to test it under different clinical conditions

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SHORT COMMUNICATION

CONTINUOUS INTRA-ARTERIAL P_{O_2} MEASUREMENTS IN INFANTS

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From the Universitats Frauenklinik Marburg BRD the Max Planck Institut fur Arbeits physiologie Dortmund BRD the Universitats Frauenklinik Rostock DDR and the Research Department 1 E blocket and the Department of Obstetrics and Gynaecology University Hospital Lund Sweden

The introduction of the Clark electrode for P_{O_2} measurements has within a few years made analysis of blood P_O a routine procedure in many hospitals However the commercially available electrodes are only suitable for samples of drawn blood Some studies have been published on intravascular P_O measurements (1, 2 3) but the electrodes have not been sufficiently reliable to warrant more than abortive attempts in humans, perhaps with one exception (3) Parker et al (4) have recently described a disposable catheter for such purposes, but no report was given about its use in humans An oxygen electrode built upon the principle of the Clark cell according to Gleichmann & Lubbers (5) but modified in size to fit the tip of a feeding tube (no 5) and with an outer diameter of 1 5 mm and 4 5 mm long was constructed by two of us (A & R Huch) The risk of coagulation was reduced by using only suitable plastic material on the exposed surface The 95% response time is 3 to 4 seconds, and the calibration is stable (Fig 1) and remains so for at least 24 hours

We consider the monitoring of arterial oxygen tension in asphyxiated infants to be a primary indication for the use of the catheter electrode but had to begin by using the oxygen electrode on a small series of 10 normal infants in order to establish its value and to learn the normal pattern of Pa_O changes The catheter was introduced 6 to 10 cm into one of the umbilical arteries within a few minutes of birth No complications were observed Pa_{O_2} increased in all infants As seen in Fig 2 it rose from 30 mmHg at 2 min 50 sec after birth to 70 mmHg 1 min later Fig 3 illustrates the rapid and reproducible increase in Pa_{O_2} after the administration of 75% oxygen by face mask The spontaneous Pa_O at 15 min after birth was in this case 85 mmHg whereas in the other cases it was about 65 mmHg at this age The attainment of a Pa_O of about 400 mmHg during oxygen mixture inhalation

Supported by a grant from the German Medical Research Council

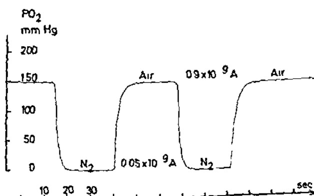


Fig 1 Calibration of the oxygen electrode in sterile water equilibrated with air and nitrogen Current readings at − 0 8 V applied voltage

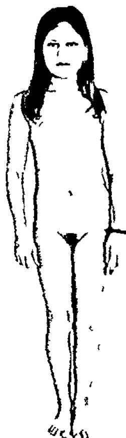


Fig 1 The patient at 13 years of age The trunk is broad and short and the nipples are placed more laterally than the normal Numerous pigmented naevi are seen The secondary sex characters are normal for her age

velopment measured by the Eklof Ringertz method (7) was retarded by 1½ years Roentgenograms of the extremities were normal including the metacarpal bones No subcutaneous calcifications were evident

Biochemical and hormone studies The concentration of serum calcium was 3 7 mEq l phosphorus 10 8 mg/100 ml and magnesium 1 4 mEq l The acid base balance in the blood was normal An Ellsworth-Howard test was performed in the patient and in another person of the same weight who served as a control (Table 1) A modified test was also performed with injection of parathyroid hormone on 3 consecutive day (Fig 2) The tubular reabsorption of phosphate was studied following PTH injection (*Table 2*) The results will be discussed later The concentration of parathyroid hormone in the serum was determined by a radioimmune assay method (Sven Almqvist Karolinska Hospital Stockholm) and was found to be less than 0 5 nanogram ml (normal range

Table 1 *Ellsworth Howard test Urinary phosphate excretion (mg P/hour) before and after administration of 200 IU of parathyroid hormone (PTH)*

	Hour	Patient	Control
Before injection	1	19 0	28 3
	2	22.1	21 1
	3	10 0	8 2
After injection	1	38 0	27 2
	2	45 5	50 2
	3	20 0	20 0
Before injection mean		17 0	19 2
After injection mean		34 5	32 5
After/before quotient		2 0	1 7

1 13-2 52 ng/ml) The PBI concentration in the serum was 6 2 μg/100 ml and TSH 31 microunits/ml (normal range 8-40) The urinary excretions of 17 ketosteroids and 17 hydroxycorticosteroids were normal for her age The serum FSH concentration was more than 5 ng/ml (increased for her age) The urinary excretion of LH was 60 U/24 hours (increased for her age) and oestron 2 9 μg/24 hours (low for her age)

Cytogenetic studies A total of 52 metaphase plates from three independent blood cultures from the patient showed two different cell populations one with 45,X chromosome cells and another with 46 chromosome cells including one structurally abnormal chromosome (Fig 3) The autoradiographic labelling

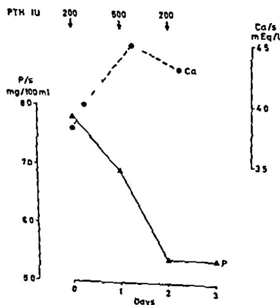


Fig 2 Changes in the serum calcium and serum phosphorus concentrations during 3 days of a PTH test

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CASE REPORT

IDIOPATHIC HYPOPARATHYROIDISM IN A GIRL WITH TURNER S SYNDROME

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Hypoparathyroidism is rare in children The symptoms are often diffuse and difficult to interpret and the diagnosis is often therefore made at a late stage We have recently examined a girl who had had vague symptoms for several years and in whom electrocardiography revealed hypocalcaemia In addition to idiopathic hypoparathyroidism she also had Turner s syndrome with coarctation of the aorta Cytogenetic examination revealed a chromosomal mosaicism not described previously

CASE RECORD

Family history The parents are both healthy and non-consanguineous The mother s first pregnancy which resulted in the birth of the present patient was uneventful Her second pregnancy from another partner resulted in a normal boy No instances of congenital malformations mental retardation parathyroid disorders or other relevant conditions have been recorded among the patient s relatives

Description of the patient H E a girl was born at term on August 6 1957 The delivery was normal Her birth weight was 2 330 g and height 46 cm At pre school age a cardiac murmur was discovered and considered to be innocent She had never shown any signs of cardiac dysfunction Her psychomotoric development was rather late Since the age of about 7 years she had been noticeably nervous and irritable She had had difficulty in concentrating at school and was easily fatigued Her school achievements had always been below average and during th last year a clear deterioration of her results had b en noted Menarche July 1970 Her menstrual periods had been irregular with short intervals rather abundant and prolonged Because of tiredness irritability and diffuse pains in the extremities advice was sought at her local hospital On examination the femoral pulses were found to be absent and when 13 $/_{12}$ years of age the patient was admitted to the ection of cardiolo y, Department of Pediatrics of the University Hospital Uppsala for further investigation Her height was 135 cm (normal ±2 S D = 155±13 cm) Her weight was 36 2 kg Her neck was short with a low hairline Her chest was somewhat broad with widely spread nipples (Fig 1) She had cubitus valgus and multiple pigmented naevi There were no signs of candida infection in the skin or nails There was no alopecia The external genitalia were normal The uterus was small and no ovaries were palpable She showed scanty pubic and axillary hair growth and slight development of the breasts (Fig 1) representing stage 3 according to Tanner (21)

Cardiovascular examination The blood pressure measured in the right arm was 155 90 The femoral pulses were not palpable ECG examination howed left ventricular hypertrophy and marked prolongation of the Q-T interval Intracardiac catheterization and cineangiography with injection into the aortic arch verified the diagnosis of coarctation of the aorta at the usual site and with a systolic pressure gradient of 60 mmHg

Neurological examination The Chvostek sign was positive EEG was pathological with general irregularity compatible with hypocalcaemia

Psychological tests revealed a mean achievement slightly below the normal for her age and marked signs of brain lesion Her achievements within the same test field varied considerably with time indicating variations in wakefulness

Dental examination showed pronounced enamel hypoplasia and blunted roots

Skeletal roentgenography Skull roentgenograms showed intracerebral calcifications The sl

Table 3 *Summary of chromosome analyses of the patient and her mother*

	Chromosomes 44	45	46	47	Total	Karyotype interpretation
Patient blood (3 cultures)		24	28		52	45 X/46 XX C t (CqXq)+
Mother blood			14		14	46 XX

the clinical picture and the presence of hypocalcaemia and hyperphosphataemia despite the presence of normal renal function the absence of malabsorption and no general skeletal disease In our patient hypoparathyroidism was first suspected from an ECG which shows the value of this examination in disturbances of the calcium balance

There are two main types of hypoparathyroidism (HP)—idiopathic hypoparathyroidism (IHP) and pseudohypoparathyroidism (PHP) Indicative of PHP apart from a positive family history are roundness of the face short metacarpal bones and subcutaneous calcifications (1 20) The diagnosis is usually decided from reaction to administration of parathyroid hormone The classical Ellsworth Howard test (8) is often inconclusive however (2 20) In our case this test showed that the patient reacted to PTH but the increase in the phosphate excretion in the urine was of the same order of magnitude as in the healthy control (Table 1) and not twice as large as can be expected in IHP or about half as in PHP (4) On modified PTH administration

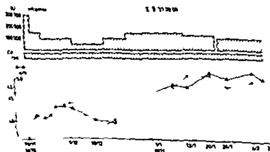


Fig 4 Serum calcium concentration and Q T interval of the electrocardiograms during

(11 20) on the other hand normalization of the serum calcium and serum phosphorus concentrations from clearly pathological values occurred as in IHP (Fig 2) The tubular reabsorption of phosphate was also normalized (Table 2) The finding of an extremely low serum level of parathyroid hormone in our patient also supported the diagnosis of IHP

Since our patient had no manifest symptoms of hypoparathyroidism until the age of about 7 years her HP may be regarded as so called IHP of later onset which is usually first expressed between the ages of 5 and 15 years (12)

The patient showed typical clinical characteristics of Turner s syndrome and an aberrant X chromosome constitution

From the cytogenetic point of view the patient exhibited mosaicism with a 45 X0 line in about half the cells and a 46 chromosome line with a structurally abnormal chromosome This aberrant chromosome was probably the result of an unbalanced translocation involving the long arms of a C and an X chromosome with extra chromosome material corresponding to the long arm of an X chromosome Only a few patients with translocations involving the X chromosome have been reported (5 9 10 13 15)

Neonatal permanent hypoparathyroidism is usually an X linked inherited disorder (17) but autosomal recessive inheritance has also been reported (16) IHP in association with Addison s disease and/or moniliasis is often familial and usually autosomally recessively inherited (19) IHP alone is a rare condition more frequently found in children than in adults Significantly more females than males are affected It is seldom familial (19) and the aetiology in unknown That IHP alone is occasionally genetically determined however is suggested by aggregation in some sibships (19)

A combination of Turner s syndrome and pseudo-pseudohypoparathyroidism has been reported in several patients (14) A ring chromosome 16 in an infant with primary hypoparathyroidism who had maldevelopment of the

Table 2 *Tubular reabsorption of phosphate (TRP) studied during 2 hours at three consecutive days after PTH injection at days 2 and 3*

IU of PTH injected	TRP
0	96 6
200	90 7
500	87 3

pattern of the DNA replication in the chromosomes (3) and the quinacrine mustard fluorescence pattern of the chromosomes (6) were studied The results of these studies indicated an X autosome rearrangement in the 46 chromosome cells where the long arm of an X chromosome was translocated to the long arm of a C group chromosome Sixteen percent of buccal mucosa cells from the patient showed one normal sized X chromatin body 84% of the cells lacked X chromatin Of 500 neutrophil leucocytes examined 1 2% had drumsticks The mother had a normal female karyotype The cytogenetic results obtained from the patient and her mother are summarized in Table 3

Other investigations Routine tests on the blood and urine gave normal results Renal function and liver function tests were normal Two-dimensional paper chromatography of amino acids in the urine showed a normal pattern Quantitative determination of the different immunoglobulins in the serum gave normal values A tuberculin test (2 TU PPD) was positive (the patient had BCG vaccination in the neonatal period) Cultures from the oral cavity and vulva for fungal infection were negative

Therapy The treatment was begun with a relatively high dose of Calciferol (300 000 IU vitamin D daily) and 1 g calcium orally each day (Fig 4) ECG and serum calcium determinations were performed at first daily and later once weekly The calcium treatment was discontinued after 3 months and only the necessary maintenance dose of Calciferol 70 000–100 000 IU daily was given The patients symptoms disappeared and her intellectual achievements improved The EEG pattern became normal Further psychological tests still showed signs of a brain lesion but there were no indications of variations in concentration or wakefulness

The coarctation was resected and the postoperative period was uneventful

DISCUSSION

In cases of hypoparathyroidism in childhood where the onset occurs after the first year of life and where there are no convulsive seizures the clinical picture is often very diffuse and difficult to interpret The diagnosis is based on

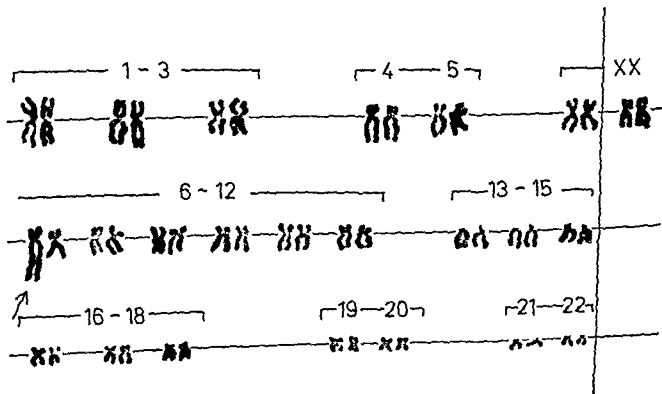


Fig 3 Karyotype of a metaphase plate from the patient s cell line with 46 chromosomes The tentative (CqXq) translocation chromosome is marked with an arrow

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CASE REPORT

BILATERAL PHAEOCHROMOCYTOMA IN TWO BROTHERS

POUL STRUNGE, HELGE M INGSTRUP JENS J LØCHTE and
CARL ZIMMERMANN NIELSEN

From the Medical Department (M) Department of Diagnostic Radiology Paediatric Department (J) and Surgical Department (K) The University Hospital Odense Denmark

Phaeochromocytoma (Ph) is a tumour arising in the chromaffin tissue of the adrenal glands or in that of sympathetic ganglion cells It is a cause of permanent or paroxysmal arterial hypertension secondary to uncontrolled re lea e of catecholamine hormones It is a rare tumour which may occur in adults as well as in children sporadically or accumulating within families Th h redity is autosomal dominant with a high (varying) degree of pene trance (3 4) Since 1947 a total of thirty families have been described Especially in the familial cases Ph may be associated with neurocutaneous manifestations (Hippel Lin dau Recklinghausen Sturge Weber Bournon ville) or it may coexist with thyroid carcinoma

Recently 2 brothers aged 15 and 7 years were treated at this hospital for bilateral Ph Since relatively few familial cases are on re cord we shall report the case histories and review the diagnostic studies as well as the preoperative and operative procedures em ployed in the management of these patients

CASE HISTORIES

Ca e 1 (M T)

A seven year-old boy who had developed normally had be n bothered during the past 3 months by pro fuse sweat ng and no turnal enuresis After having sustained a head injury h was transferred from a local hospital to the Department of Neurosurgery of this hospital n the suspicion of increased intracranial pre sure BP 210 170 mmHg) At that time he was awake rational pale and with severe headache It was concluded from the investigations that he had a hypertensive encephalopathy of not quite recent origin Three days later he had a generalized seizure and was transferred to the Paediatric Department Antihyper tensive treatment intravenously was instituted and BP fell in the course of a few hours from 230 190 to 180 120 and the boy regained consciousness

The diagnosis of Ph was suspected and confirmed by determination of urinary catecholamines and vanil lyl mandelic acid (VMA) which were 2 800 μg/24 h (normal < 100) and 57 mg/24 h (normal < 8) re spectively Intravenous pyelography showed no dis placement of the p lvis or calyces but a subsequent aortography with elective renal arteriography dis closed a well-defined mass in the right adrenal (Fig 1) No similar tumours could be found in the left adrenal chest or pelvis

Treatment was now started with phentolamine but later adjusted to bensylyte (phenoxybenzamine) 20 mg × 4 daily and propasylyte (propranolol) 5 mg × 4 daily BP was reduced to 160 100 with minor orthostatic complaints.

After 3 weeks pre treatment an operation was car ried out according to the principles described by Able (1) Through a transabdominal incision a well defined tumour (3 × 4 × 5 cm) was removed Intra abdominal palpation did not give rise to any sus picion of remaining tumours During the operation BP fluctuated (120–200/100–140) but after exci sion of the tumour it dropped to 110 80

Shortly after this operation BP rose again (170/ 130) and antihypertensive treatment was re instituted Determination of the urinary excretion of catechol amines and VMA showed 2 100 μg/24 h and 22 mg/ 24 h A new renal arteriography revealed at this time a tumour in the left adrenal

Re-operation was performed 5 weeks after the primary procedure and a tumour (3 × 3 × 3 cm) was extirpated from the left adrenal After removal BP fell to 90/70 but was stabilized by metaradrine (me taraminol) intravenously

facial and neck structures including the parathyroids has also been reported (18) A combination of Turner s syndrome with aberrant X-chromosome constitution and IHP however, has not been reported earlier, to our knowledge Results of cytogenetic investigations on further patients are required to ascertain whether there is an etiological connection between chromosomal aberrations and IHP

SUMMARY

Idiopathic hypoparathyroidism (IHP) was diagnosed in a 13 year old girl with symptoms since the age of 7 years The disease was suspected from an ECG and the diagnosis of IHP was based on the clinical picture, the reaction to administration of parathyroid hormone and the serum parathyroid hormone concentration She had typical features of Turner s syndrome and coarctation of the aorta Cytogenetic investigation showed two different cell populations—one with a 45 X karyotype and another with 46 chromosomes including a tentative X-autosome translocation The possible relationship of the hypoparathyroidism to the chromosomal aberration is discussed

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few months There is no doubt that the cranial injury and the neuroradiological investigations accelerated the development of his disease In children the course is often rapid (4) and the condition may be provoked or accelerated even by minor trauma (7) The older brother had symptoms for many years was small for his age and the diagnosis was made incidentally This boy appears to have had permanent hypertension without paroxysmal exacerbations which occurs in 90 of paediatric cases (7 13)

Both boys had reddish blue scaly cutaneous and reversible changes on the hands Similar changes have been described by others (7 9 13)

In the preoperative medical management of Ph it is endeavoured to reduce the blood pressure to a stable lower level Alpha receptor blockade is the main component of this treatment Phentolamine is best suited for acute lowering of BP whereas bensylyte must be considered better suited for long term pretreatment However several authors have reported difficulties in attaining the desired lowering of BP so that supplementary treatment by beta blocking agents has been used to an increasing extent (2 5 10 12) also because beta blockade protects from tachycardia and arrhythmia

In our cases these drugs gave a stable BP so diagnostic and therapeutic procedures could be performed Judging by the experience of others and our own the preoperative medication should not be standardized owing to variation in tolerance

In the earliest studies on Ph the radiological investigations often consisted in pyelography and retroperitoneal air insufflation Most authors were reserved in using aortography because this procedure often gave rise to hypertensive crisis Today after the introduction of the blockade therapy the risk of this complication to aortography has been considerably reduced (6)

The purpose of aortography is to ascertain the presence of adrenal or extra adrenal tumours and their blood supply which is of great importance in the surgical procedures (8 11 14)

This angiography is more important in children where 30% (against 4% in adults) have bilateral occurrence of Ph and in familial cases 50% of patients have bilateral Ph (4 7, 13) In our case 1 neither the first angiography nor the operative palpation could reveal the tumour which was later demonstrated in the left adrenal Nor could the characteristic fall of BP after ligation of the tumour vessels be taken to be a reliable criterion that no tumour tissue had been left behind

The risk involved in the surgical procedure is hypertensive crisis and cardiac arrhythmias while the tumour is being detached and hypotension when the vessels are ligated In the present cases the alpha and beta blockers could not eliminate fluctuations in BP but no hypertensive crisis or cardiac arrhythmias occurred, and the hypotensive episodes were easily counteracted by infusion of a vasopressor agent

In a material of 100 children with Ph (13) the total operative mortality was 22% (45% prior to 1954 13% after 1954) Presumably a considerably increased operative risk has still to be expected in children with bilateral Ph as compared with those having unilateral Ph (7) Greater materials with pre treatment after modern principles (alpha beta blockade) are not yet available

SUMMARY

Two cases of bilateral phaeochromocytoma in brothers are described In the younger of the two brothers the symptoms were of acute onset after a cranial injury whereas in the older brother the disease had run a slower course and was detected incidentally The bilateral tumours were located by selective arteriography (in the former case after repeated investigation) which could be performed without major risk to the children after effective treatment of the hypertension had been instituted using alpha and beta adrenergic receptor



Fig 1 Selective renal arteriography on the right visualizing the phaeochromocytoma (case 1)

The postoperative course was uneventful and at follow up 3 months later the patient was fit had normal BP optic fundus and excretion of catecholamines VMA and 17 hydroxycorticosteroids

Case 2 (S T)

A 15 year old brother of M T For many years this boy had suffered from hyperhidrosis hot flushes reduced tolerance of heat transient diffuse headache and dry scaly skin and red fingers and toes When his brother was admitted their mother realized that the two boys symptoms were of the same kind

At admission the boy was found to be of delicate build and he had not yet entered pub rty Height and weight (146 cm/35 kg) corresponded to an age of 11–12 years

Investigations BP 140/115 mmHg Urinary excretion of catecholamines 2 100 μg 24 h (normal < 100) and of VMA 29 mg/24 h (normal < 8) Ophthalmoscopy Grade II hypertensive angiopathy

Aortography and elective renal arteriography revealed a well defined adrenal tumour on both sides (Fig 2) In the chest and pelvis no tumours were found

The patient was treated with phentolamine 20 mg × 3 daily and propasylyte 10 mg × 2 daily On this medication his BP dropped to normal and there were but mild orthostatic complaints

At op ration a 3 × 4 × 5 cm well defined tumour was removed from the right adrenal gland and there after a 2 × 2 × 3 cm tumour from the left adrenal During the operation BP fluctuated initially around 150–200/110–150 but after the removal of th right sided tumour it fell abruptly to 110/80 On excision of the left sided tumour there were repeated episodes of hypotension (90/70) which was treated with metaradrine Moreover a blood transfusion of 375 ml was given as the blood loss amounted to 300 ml

The postoperative cour e was uncomplicated and at follow up 3 months later BP optic fundus as well as urinary catecholamines VMA and 17 hydroxy corticosteroids were normal He no longer suffered from excessive sweating and cutaneous changes on the fingers had disappeared

For all the anaesthesias the boys had premedication with atropine pethidine and the anaesthesias were p rformed with halothane/nitrous oxide/oxygen/gallamine

Histological examination of the four glands showed (benign) Ph

Family investigation

The closest kin were asked to join a clinical investigation but refused on grounds of principle The father (54 years old) and two brothers (22 and 19 years old) had normal excretion of catecholamines and VMA and their BP was like their mothers, found normal

On questioning the parents and the practitioner we were informed that no case of neurocutaneous diseases hypertension or thyroid disease had occurred either in the father s or in the mother s large family

DISCUSSION

The symptoms and signs of phaeochromocytoma (Ph) are much varied and even in classical cases the diagnosis is often greatly delayed in children (4) as the reported histories illustrate The younger brother had in retrospect experienced symptoms for only a



Fig 2 Aortography showing bilateral phaeochromocytomas (case 2)

PROCEEDINGS OF PAEDIATRIC SOCIETIES

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Meeting February 26 1972

…entz, H. Feychting P Herin B Lager- …t K Thodenius & E. Enocksson *Panel …ussion Transfer of newborns to a referral …pital—Experiences and practical advice*

… Bodegard & R Zetterström *Diagnostic and …rapeutic problems in children with PKU … galactosemia*

K. Hall P Olin & B S Lindblad *Growth hormone and sulphation factor—clinical experiences*

U Berg *Renal function in children with recurrent urinary tract infections*

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… Wendell *666 umbilical artery catheteriza…ns in neonates*

LEUKOCYTE FUNCTION AND RESISTANCE TO INFECTIONS

… Bjorksten *Some method variations in NBT …st*

…he histochemical NBT test of Park et al (1) …as proved valuable in differential diagnosis …f infectious diseases Since only 0 5 ml of …lood is required an attempt was made to …valuate the use of capillary blood in the test …t was found however that the NBT reducing …apacity of the granulocytes is significantly …ower in capillary than in venous blood samples …rom 57 patients with different infectious dis …eases. This discrepancy between venous and …apillary blood made us investigate whether …erum factors might influence the NBT reduc …tion test Granulocytes in venous blood sam …pl s from 21 healthy volunteers were tested for …heir NBT reducing capacity spontaneous and …a ter endotoxin stimulation Granulocytes …ashed and resuspended in saline with 0 2% glucose can hardly be activated while those resuspended in fresh pooled human serum can although not to the same extent as in whole blood If inactivated serum is used the granulocytes can be only slightly stimulated

Investigations to ascertain which serum factors influence the NBT test are in progress

Reference

Park, B H Fikrig S M & Smithwick E M *Lancet* 532 1968

K M Lundmark *Familial granulocytophenia of unknown etiology*

A family is described in which three children of four one boy and two girls suffer from an increased susceptibility to staphylococcal infec tions mainly in the skin whereas the fourth sibling a girl appears healthy as do the parents The affected children have a normal response to viral infections and to immuniza tion with viral agents The principal findings in the investigations of their immune defences were the following

1 *Inflammatory response* Peripheral blood

blockers During continued blockade the adrenal tumours were removed surgically (in the younger brother in two stages) without complications

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1 *Inflammatory response* Peripheral blood

neutropenia and eosinophilia Normal number of monocytes Bone marrow normal except for a low proportion of segmented neutrophils Adrenaline response Weak? Skin window Late response and mainly monocytes Eosinophils increased compared with controls Phagocytosis normal Bactericidal capacity (including NBT test) normal Complement Total and C 3 normal

2 *Plasma cell function* Immunoglobulins IgG normal, IgA elevated IgM and IgE varying levels Antibody rise after vaccination normal Isohemagglutinins normal ASTA Increase in only one of the patients

3 *Lymphocyte function* Lymphocytes in peripheral blood normal Lymphoid tissue sparse, slight local response to infection Biopsy (one patient) a few germinal centres Many plasma cells Tuberculin test negative in spite of neonatal BCG vaccinataion DNCB test (one patient) weak, positive reaction The main defect appears to lie in the primary inflammatory response although the lymphocyte function demands further investigation in all siblings The granulocyte function will be further investigated, especially with regard to interactions with complement There may be a combined defect in the immune defences The condition appears to be inherited in an autosomal recessive manner

WEIGHT REDUCTION IN TEEN AGE GIRLS

I Nylander and N G Holmberg *Weight reduction and psychological problems in teen agers with amenorrhea*

K Schleimer *Preliminary results from a study of teen agers who claim to have reduced weight*

L Beckman & N Myrberg *Cheilognathopalatoschisis in northern Sweden—A genetic study*

I Carlsson, H Grahnén & G Jonsson *The interaction between diet bacterial flora of the oral cavity and caries—A preliminary report*

G Samuelson L Hambraeus & G Holmgren *Dietary treatment of adult patients with phenylketonuria*

A pilot study and a subsequent double blind study have been performed on 7 previously untreated elderly phenylketonuria patients in order to evaluate the effect of phenylalanine deficient diet The effect of the treatment was evaluated by the observation of 6 psychiatric symptoms according to a 5 grade scale Good and/or rather good effect of the treatment was noticed in 3 of the patients, while showed only a slight effect during the pilot study In a subsequent double blind study of the same patients no effect was seen of the treatment with phenylalanine-deficient diet *The necessity of double blind studies in investigations of this kind is stressed*

ASPECTS OF BREASTMILK

P G Bergfors *When and why do mothers stop breastfeeding?*

An investigation made in the city of Skellefteå in Northern Sweden during 1967–68 shows a rapid decrease in the incidence of breast feeding among mothers of this region The wholly breast fed infants had at 4 weeks of age decreased from 87 to 52%, at 2 months to 28 at 3 months to 15 and at 4 months to 7%

Variations in the incidence of breast feeding between different parts of the region seemed to depend more on the time and interst the district nurse had for the mothers at home and her attitude to breast feeding than on the type of the population (rural/urban etc) The married women breast fed longer than the unmarried ones as did women in the age group of 30–35 compared with the younger and the older mothers The third child of the family was nursed longer than the older and younger siblings The volume of milk produced by the mother on the day before discharge from the hospital seemed to be the decisive factor with regard to the duration of breast feeding

J Winberg & G Wessner *How often is cow s milk needed in a nursery?*

L Gothefors J J Gindrat L A Hansson & J Winberg *E coli antibodies of human milk and stools—a part of the defence mechanism against infection in the newborn infant?*

In most animals the fetus and the newborn receive their antibodies from the mother passively In man this transfer takes place via the placenta The extra uterine route colostrum and milk is essential to many of our domestic animals where the antibodies are readily absorbed from the digestive tract This is not possible in man

Yet human colostrum contains gammaglobulin mainly secretory IgA and the antibodies of this fraction are able to survive a passage through the gastrointestinal tract This fact points to the possibility of these antibodies having a local protective effect

In a preliminary study we were able to demonstrate high titres of antibodies against the 0 antigen of some of the most common serotypes of *E coli* Infants with adequate intake of breast milk with these high titres also had such antibodies in their stools

Possible mechanisms for the IgA local antibodies are

(*a*) The antibody is a typical antibacterial one

(*b*) The antibody is directed against the gut epithelial cells forming a cover preventing cell invasion

(*c*) The antibody might be protective for the lactobacillus giving them a possibility to be established in the gut

(*d*) The antibody could be directed against different toxins

More detailed studies are necessary to understand the function of colostral IgA

J Gentz

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NEW BOOKS RECEIVED

F E Camps & R G Carpenter (eds) *Sudden and unexpected deaths in infancy (Cot Deaths)* Proceedings of the Sir Samuel Bedson Symposium Addenbrooke s Hospital Cambridge April 17-18 1970 129 pp illus John Wright & Sons Ltd Bristol 1972 £2 00

G J Hill *Leprosy in five young men* 204 pp illus Colorado Associated University Press Boulder *1971 $8 00*

R S Illingworth *The treatment of the child at home A guide for family doctors* 301 pp Blackwell Scientific Publications Oxford and Edinburgh 1971 £2 75

I M Nilsson (ed) *Blodnings och trombossjukdomar* 275 pp illus Almqvist & Wiksell Stockholm 1972 Skr 75 -

E S Sachs *Trisomy G/normal mosaicism, VII A cytological and clinical investigation* 93 pp illus H E Stenfert Kroese N V Leiden 1971 D Fl 19 23

P H Jongbloet *Mental and physical handicaps in connection with overripeness ovopathy* 147 pp illus H E Stenfert Kroese N V Leiden 1971 D Fl 24 -

P J Huntingford R W Beard F E Hytten & J W Scopes (eds) *Perinatal medicine* Proceedings of the 2nd European Congress of Perinatal Medicine London April 1970 378 pp illus S Karger AG Basel 1972 sFr 80 -

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ANNOUNCEMENT

THE INTERNATIONAL SOCIETY FOR PAEDIATRIC NEUROSURGERY

On may 26 1972 The International Society for Paediatric Neurosurgery was formed The pro tem officers are Dr Jacques Rougerie (President) Dr Anthony J Raimondi (Secretary) and Dr Joseph Ransohoff (Treasurer) Requests for membership should be directed to Docteur Maurice Choux Departement de Neuro Chirurgie Centre Hospitalier et Universitaire Hopital Nord Marseille 15 France

The first scientific meeting will be held in conjunction with the World Federation of Neurosurgical Societies in Tokyo Japan in 1973 For details concerning the meeting write to Doctor Satoshi Matsumoto Kobe University Chairman Dept of Neurosurgery 12 Kusunoki cho 7 Chome Ikuta ku Kobe Japan